Oral HPV Infection and Head and Neck Squamous Cell Carcinoma in HIV-infected and HIV-uninfected Individuals

by
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Abstract

Background: Oral HPV infection is etiologically associated with a subset of Head and Neck Squamous Cell Carcinomas (HNSCCs). However, the natural history and risk factors of this infection are largely unexplored particularly in high risk groups such as HIV-infected individuals.

Objectives: This dissertation aims to examine the natural history and risk factors for oral HPV infection and HPV-related HNSCC in HIV-infected and HIV-uninfected individuals.

Methods: We utilized three different longitudinal studies involving HIV-infected individuals. We semi-annually collected oral rinse samples (in the first and second studies) and anal swabs (in the first study) and analyzed them for 37 different HPV DNA types utilizing the Roche Linear array. In the third study, HNSCCs were validated through chart review or through cancer registry-linkage. In each study, we collected information on biologic and behavior risk factors. We analyzed data utilizing 1) Kaplan-Meier survival analysis, 2) Wei-Lin-Weissfeld regression, and 3) Mixed effects Poisson regression.

Results: Study 1: Among HIV-infected individuals, the prevalence (84% vs. 28%), incidence (aHR=4.7, 95%CI=3.6-6.2) and persistence (aHR=1.5, 95%CI=1.2-1.9) were all significantly higher for anal versus oral HPV infections, respectively.

Study 2: While 28% of HIV-infected and at risk HIV-uninfected participants had at least one type-specific incident oral HPV infection within 24 months, only 7% of incident oral HPV infections were persistently detected for two or more years. Oral sex and immunosuppression were associated with increased risk of oral HPV infection, while male gender, older age, and current cigarette smoking were associated with increased persistence.

Study 3: HPV-related HNSCC is three times more common in HIV-infected individuals

compared to the general population. HNSCC was associated with reduced CD4 measured prior to cancer diagnosis.

Conclusion: Oral HPV infection is regularly detected in HIV-infected individuals, but commonly clears and HPV-related HNSCC is only modestly elevated in HIV-infected individuals compared to the general population. Older age, gender/sexual orientation, cigarette smoking, and oral sexual behaviors all appear to impact the natural history of oral HPV. HIV-related immunosuppression appears to have the strongest impact in the acquisition or re-activation of oral HPV, but may have a modest role throughout the oral carcinogenesis process.

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Chapter 1: Introduction

Note:

The following chapter is modified from a paper published in the "Current Opinions in Oncology" in September 2013 (Beachler et al, 2013 Sept, 25(5):503-11).

Introduction:

Human Papillomavirus (HPV) infection, a commonly detected DNA virus widely known as the necessary cause of cervical cancer,[1] has been established as a major etiologic factor for head and neck squamous cell carcinoma (HNSCC).[2] Research suggests that HIV-infected individuals are at higher risk for oral HPV infection and HPV-associated HNSCC.[3,4] In this introductory chapter, we review the literature on the risk factors and burden of oral HPV and HPV-associated HNSCC among HIV-infected individuals, discuss cancer prevention possibilities, and suggest future research directions.

HPV and Head and Neck Squamous Cell Carcinoma Overview:

HNSCC is a heterogeneous group of cancers that includes cancer of the oral cavity pharynx, and larynx, and is the one of the ten most common cancers worldwide with an annual incidence of over 300,000.[5] HPV is known to cause a subset of HNSCCs, with HPV-associated HNSCCs having distinct genetic, clinical, and epidemiological characteristics from HPV-unassociated HNSCCs.[6] HPV-associated HNSCCs represent approximately 25% of all HNSCCs in the general population,[7,8] and usually arise in the oropharynx, which includes the base of the tongue and the lingual and palatine tonsils.[9] These cancers are independently associated with sexual behavior including recent and lifetime number of oral sex partners.[6] In

contrast, the majority of HPV-unassociated HNSCCs occur in the oral cavity and larynx and are primarily associated with tobacco and alcohol use.[6]

Control of the cell cycle is impacted in both HPV-associated and HPV-unassociated cancers. HPV-associated HNSCCs involve E6 and E7 oncogene expression, which functionally inactivate tumor suppressor genes p53 and pRB, while HPV-unassociated HNSCCs mutationally inactivate p53 and p16, which can lead to unregulated cell growth.[10,11] HPV-associated HNSCCs, which now account for many of the HNSCCs diagnosed before age 60, have shown better response to chemotherapy and radiotherapy, and improved survival compared to HPV-unassociated HNSCCs. [12,13]

The incidence of HPV-associated HNSCC has increased in the general population of many developed countries over the past several decades.[14,15] This increase could be explained by generational differences in sexual behavior or increased persistence or progression of oral HPV due to changes in co-factors. Despite this increasing trend of HPV-associated HNSCC, the natural history of oral HPV has been largely unexplored.

Oral HPV infection among HIV-infected individuals:

While the natural history of oral HPV has been largely uninvestigated, several cross-sectional studies have observed that HIV-infected individuals have a 2-3 fold higher odds of prevalent oral HPV infection compared to HIV-uninfected individuals, even after adjustment for sexual behavior and other relevant factors.[3,16] Recent studies suggest HIV-infected individuals have an overall oral HPV DNA prevalence between 20% and 45% (in the alpha genus), and an oncogenic oral HPV DNA prevalence between 12% and 26%, (Table 1.1).[3,16,17] HPV16, which causes more than 80% of HPV-associated oropharyngeal cancers,[7,18] is the most commonly detected oral HPV type in HIV-infected individuals with a

prevalence around 2-6%.[16,19-21] In contrast, a study utilizing a representative population of the United States (US) found that 7% of healthy adults have a detectable oral HPV infection, while about 1% have detectable oral HPV16.[22] Estimates of oral HPV prevalence among HIV-infected individuals likely vary because of multiple factors including differences in sample collection, processing, number of HPV types tested, DNA detection methods, and the study participant characteristics (Table 1.1). While oral HPV prevalence is considerably lower than prevalence of anogenital HPV infection in HIV-infected and HIV-uninfected individuals,[23] it is unclear if this is due to reduced incidence or persistence of oral as compared to anogenital HPV infection.

Head and neck squamous cell carcinoma in HIV-infected individuals:

The risks of several different cancer types are elevated in HIV-infected individuals due to behavioral and biological characteristics, immunodeficiency, and potentially chronic inflammation and immune dysfunction/senescence.[24] Indeed, HIV-infected individuals in developed countries have a modestly increased risk of both HPV-associated and HPV-unassociated HNSCCs compared to the general population. AIDS-cancer registry match studies have found that the standardized incidence ratios (SIRs) for HNSCC are between 1.5 and 4 fold higher among HIV-infected individuals compared with the general population (Table 1.2).[4,25-29]

HIV-infected individuals are also at increased risk of laryngeal, oral cavity and other HPV-unassociated HNSCCs,[4] which is likely due to their high prevalence of tobacco use,[30] the strongest risk factor for these cancers.[31] While these registry match studies are not typically adjusted for potential confounders other than age, one recent study that did control for risk factors including tobacco and alcohol use found a non-significant overall increased

risk of oral cavity/pharynx cancer for HIV-infected as compared to HIV-uninfected individuals in California (aRR=1.4, 95%CI=0.9-2.1).[29]

While most studies have considered all HNSCC sites together, several registry-match studies attempted to quantify the risk of HPV-associated HNSCC by exploring cancer risk in specific anatomical subsites. These studies reported a 1.5-4 fold higher risk of oropharyngeal or tonsillar cancer for HIV-infected individuals compared to the general population (Table 1.2).[4,25,28,32] While estimates suggest that half or more of oropharyngeal cancers are HPV-positive in the general population,[14,15] the proportion of HPV-positive oropharyngeal tumors in HIV-infected individuals is unknown. While their exact level of risk is unclear, HIV-infected individuals appear to be at moderately increased risk of HPV-associated HNSCC compared to the general population based on the modestly higher oropharyngeal cancer incidence. Interestingly, the magnitude of this increase (SIR~1.5-4) is lower than other HPV-associated cancers such as anal, cervical, vulvar, and penile cancer which each have SIRs of 5 or greater.[4,28]

Impact of Sexual Behavior:

An increased number of oral sexual partners is a risk factor for both oral HPV infection[16,33] and HPV-associated HNSCC.[6] Given the high number of lifetime sexual partners among many HIV-infected individuals,[16,34] one might expect the incidence of HPV-associated HNSCC among HIV-infected individuals to be higher than what is currently observed; particularly among the most sexual active groups such as men who have sex with men (MSM).[16,35] However, several registry based studies have found a non-significantly *lower* incidence of oropharyngeal cancer in HIV-infected MSM compared to HIV-infected injection drug users and heterosexual men (Table 1.2).[25,26,28] One potential explanation is that

the probability of acquiring oral HPV from performing oral sex on a man (fellatio) may be lower than when performing oral sex on a woman (cunnilingus). While studies have not extensively compared HIV-uninfected MSM and men, two large studies recently suggested that oral HPV DNA prevalence[22] and HPV-associated oropharyngeal cancer[14] are considerable higher in males compared to females in the US general population.

There are at least two hypotheses as to why oral HPV may be more transmissible when performing oral sex on women. One hypothesis is that the female genital region may have a greater HPV viral load than the male genitals,[36-39] despite similarities in genital HPV DNA prevalences,[40-42] and that this higher viral load could increase the likelihood of oral HPV acquisition. In contrast, a second hypothesis suggests that the keratinized epithelium from male genitals may be less likely to induce an immune (antibody) response than mucosal surfaces such as the cervix or the anal canal.[43,44] Thus, the high level of natural antibodies developed after a cervical or anal HPV infection in women and MSM might conceivably be more likely to protect them from acquiring subsequent oral HPV infections.[43,45-47] Further investigation is necessary to explore these hypotheses.

These hypotheses coupled with the demographics of the HIV-epidemic in developed countries may in part explain the moderate risk of HPV-associated HNSCC seen in the HIV-infected population. In the US, approximately half of HIV-infected individuals are MSM, while a little over a quarter are women (who are almost all heterosexual).[48] This suggests that the proportion of heterosexual males (who perform oral sex on women) is lower among the HIV-infected population than the general population. If the risk of acquiring oral HPV infection is truly highest among heterosexual males, then this could help

explain the relatively moderate risk of HPV-associated HNSCC (compared to other HPV-associated cancers) seen in HIV-infected individuals in developed countries.

Impact of Immunosuppression and Antiretroviral Therapy (ART) Use:

HIV-related immunosuppression may be a strong risk factor for oral HPV incidence or persistence given the 2-3 times higher adjusted odds of oral HPV prevalence in HIV-infected individuals compared to HIV-uninfected individuals.[3,16] Advanced stage of HIV disease, characterized by low CD4 T cell count and high HIV RNA viral load, has also been associated with increased oral HPV prevalence which may reflect a loss of viral control in those with compromised immune systems (Figure 1.1).[3,16]

The direct effect of immunosuppression on oral HPV persistence and HPV-associated HNSCC is currently less understood, but research on other HPV-associated cancers suggest immunosuppression may act more on the *earlier* stages of the HPV carcinogenesis process.[25,49] Oral cavity/pharynx cancer is elevated among both HIV-infected individuals and solid organ transplant recipients (another immunosuppressed population) suggesting a potential link between immunosuppression and HPV-associated HNSCC.[50] In addition, three studies have found that the incidence of oral cavity/pharynx cancer was higher, although not significantly so, among those with a reduced CD4 T cell count.[26,29,51] Engels et al also observed a higher risk of oral cavity/pharynx cancer in individuals with AIDS relative to HIV-infected individuals who have not developed AIDS.[51] However, another recent study suggested reduced CD4 at AIDS diagnosis was associated with a *reduced* risk of oropharyngeal cancer among patients 28-60 months after AIDS offset.[28] One explanation for the heterogeneity of these results could be that a higher proportion of HPV-unassociated HNSCCs occur in certain populations, as HPV tumor status has not been

explored and HPV-associated and unassociated HNSCC might be differentially related to immunosuppression. These registry based studies also lack detailed covariate information such as sexual behavior and smoking status and cannot comprehensively evaluate the effect of cumulative and recent immunosuppression.

Effective antiretroviral therapy (ART, also known as HAART) has greatly improved the life expectancy of HIV-infected individuals while reducing viral-related malignancies such as Kaposi Sarcoma and Non-Hodgkin's lymphoma.[52] However, the incidence rates of HPV-associated malignancies have remained stable in the ART era, or have increased in the case of anal cancer. A preliminary study suggested ART use was associated with increased six month oral HPV persistence,[53] and other studies have suggested ART use is associated with an increase in oral lesions/warts.[54,55] However, these studies may be prone to confounding by indication, as ART is more likely to be indicated for sicker individuals.

The role of ART on cervical HPV and related squamous epithelial lesions (SILs) has been more extensively explored with the majority of well-designed studies suggesting a benefit.[56-58] While some of the initial studies suggested a similar cervical HPV persistence and progression of SILs comparing ART users and non-users,[59,60] more recent reports suggest ART reduces the incidence of cervical HPV,[57] decreases the incidence of squamous epithelial lesions[56,58] and increases the regression of these lesions.[56,57] However, if ART use does not fully recover oral HPV-specific immunity it may not be able to substantially modify the elevated oral HPV incidence or persistence seen in HIV-infected individuals. Therefore, HPV-associated HNSCC could pose an increased threat for immune-competent HIV-infected individuals, if ART improves survival but did not improve control of oral HPV infections.

Tobacco use and other co-factors for HPV-associated HNSCC:

Although HPV-associated HNSCC has often been described as a cancer among non-smokers and non-drinkers, there is growing evidence that tobacco may play a substantial role in the development of some of these cancers.[61] Tobacco use is an established risk factor for cervical cancer,[62] and is associated with oral HPV prevalence [22,33,63,64] and six month oral HPV persistence[23] in HIV-infected and HIV-uninfected individuals. Other studies have shown tobacco use can reduce the innate and cell-mediated immunity at the systemic level and in the local oral region[65,66], suggesting an immunosuppressive effect of tobacco.

The direct role of tobacco on HPV-associated HNSCCs is less clear as several studies have found an association with increased risk,[18,67-69] while others have not.[6,70] This question is particularly important for HIV-infected individuals, as the prevalence of tobacco use in this population is considerable higher than the general population with estimates suggesting 40-70% of HIV-infected individuals in developed countries may be current smokers.[30,71]

There are several other factors being investigated that may increase the risk of HPV-associated HNSCC including marijuana and alcohol use. While there is a lack of data on these factors in HIV-infected individuals, epidemiologic studies among HIV-uninfected individuals have found inconsistent results between risk of HPV-associated HNSCC and both marijuana [6,72-74] and alcohol use [6,67] as some have observed an association with increased risk and others have not.

Future steps and challenges

While prevention of HPV-associated HNSCC is a developing research area, screening for HNSCC remains a challenge. A recent study found that feasibility of an oral Pap smear equivalent remains poor in HIV-infected individuals, as the limited number of the observed cytopathologic abnormalities was not associated with HPV16.[75] Fakhry et al suggest that this potential screening tool may not be feasible due to anatomic sampling limitations and the relatively low incidence of HNSCC.[75]

Although prophylactic vaccination is an effective tool to prevent other HPV-associated cancers, its efficacy in preventing HPV-associated HNSCC among HIV-infected individuals has not yet been evaluated. The recently developed HPV vaccines are likely to have the potential to protect against HPV-associated HNSCC, considering they include prevention of HPV16, which accounts for over 80% of HPV-associated HNSCCs.[7,8,18] While rigorous efficacy studies have not been performed, initial observational data suggest the vaccine may provide protection against prevalent oral HPV16 and HPV18 in young HIV-uninfected women.[76] The quadrivalent vaccine has been shown to be safe and immunogenic among HIV-infected individuals,[77,78] and is recommend by the American Council on Immunization Practices for HIV-infected individuals aged 11-26.[79] Efficacy studies for anogenital HPV are currently being performed in older HIV-infected individuals.[80]

There are still several other unknowns regarding oral HPV infection and HPV-associated HNSCC among HIV-infected individuals. First, the proportion of HNSCCs cancers caused by HPV among HIV-infected individuals is currently unknown and would help to understand its etiology and identify who is at the greatest risk for this disease. In addition, the natural history of oral HPV is unstudied, and the incidence and persistence rates or oral HPV infection is undefined in both HIV-infected and HIV-uninfected

individuals. It is not clear what factors increase the risk of oral HPV incidence, persistence and progression to subsequent HNSCC. The relative effects of HIV, reduced immunity, tobacco use, sexual behavior and other factors on the natural history of oral HPV are still not well understood. Long term longitudinal studies are needed to further explore these factors and how they differ between HIV-infected and HIV-uninfected individuals.

Conclusion:

HIV-infected individuals are living longer and therefore may have the opportunity to acquire more slowly developing HPV-associated malignancies. Indeed, HIV-infected individuals currently appear to have a moderately increased risk of HPV-associated HNSCC, which might potentially increase as this population ages. In addition, HIV-infected individuals have many of the potential risk factors for this disease (immunosuppression, increased sexual partners, tobacco use) and thus should be further studied and considered for future potential preventative measures.

Studies involved in the dissertation:

Data from several prospective cohort studies involving HIV-infected individuals is utilized in this dissertation (Table 1.3). Chapter 2 uses the Human Oral Papillomavirus Etiology (HOPE) Study conducted at the Johns Hopkins Hospital's Moore Clinic in Baltimore, Maryland. For this study, 404 HIV-infected participants volunteered oral and anal specimens for HPV detection semi-annually between 2006 and 2010. Serum, demographic and risk factor information were also collected from this population at baseline.

Chapter 3 utilizes data from the Persistent Oral human Papillomavirus Study which includes 1,230 men from Multicenter AIDS Cohort Study (MACS) and women from

Women's Interagency HIV Study (WIHS).[81,82] Both the MACS and WIHS include semi-annual follow-up of HIV-infected and at risk HIV-uninfected individuals and these studies are especially known for the strong retention rates.

In chapter 4, data from the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) was utlizied.[83] Our study within the NA-ACCORD includes individuals from 17 interval and clinical studies, and is one the first to explore Head and Neck Squamous Cell Carcinoma (HNSCC) in HIV-infected individuals. The analysis involved 82,375 HIV-infected individuals and included 248 cases of HNSCC.

Specific Aims:

The specific aims of this dissertation are as follows:

Aim 1 – Compare the natural histories of oral HPV and anal HPV infection among HIV-infected women, heterosexual men, and men-who have sex with men

Hypotheses: 1. Incidence of anal HPV infection is higher than oral HPV, but the persistence of both infections is similar

2. After adjusting for the number of sexual partners, heterosexual men have a higher risk of incident oral HPV but a lower risk of incident anal HPV compared to women and men-who have sex with men.

Aim 2a– Evaluate the effect of HIV and related immunosuppression on newly detected and persistent oral HPV infection in the Multicenter AIDS Cohort Study (MACS) and Women's Interagency HIV Study (WIHS).

Hypothesis: Reduced current CD4 T cell count and reduced nadir CD4 are independently associated with persistent oral HPV infection.

Aim 2b – Evaluate the effect of other risk factors on newly detected and persistent oral HPV infection in the MACS and WIHS

Hypothesis: Current tobacco use increases the susceptibility of acquiring oral HPV infection when exposed and reduces the ability to clear or control oral HPV infections. HIV-infection modifies these relationships.

Aim 3 –Examine the incidence, calendar trends and risk factors (particularly immune status) for HNSCC in a large consortium of HIV-infected individuals in North America (NA-ACCORD).

Hypotheses: 1. The incidence of HPV-related HNSCC is elevated in HIV-infected compared to HIV-uninfected individuals and has been increasing over the past decade among HIV-infected individuals
2. Tobacco use and reduced immune status are risk factors for HPV-related HNSCC.

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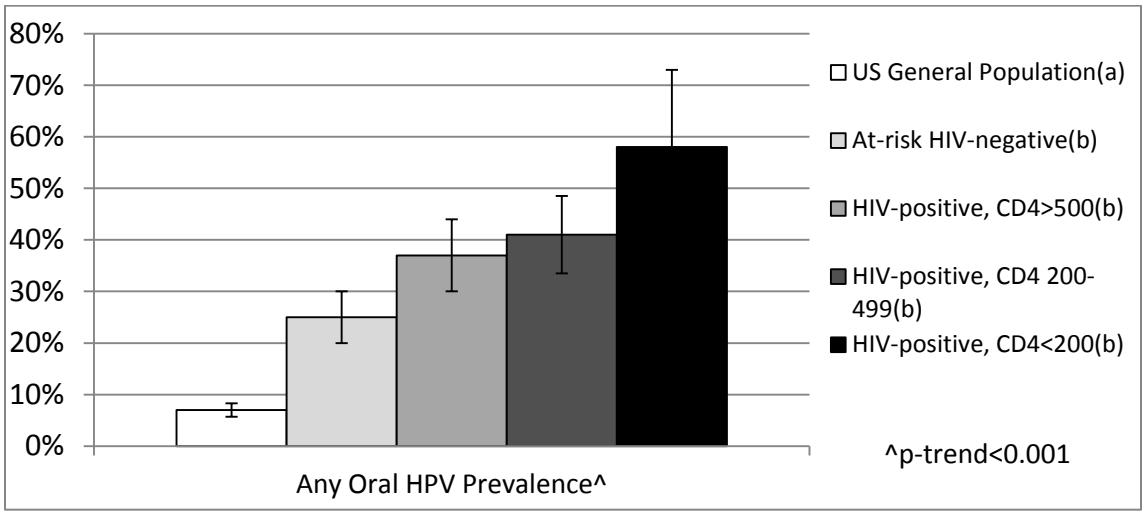
Figure 1.1: Impact of immunosuppression on oral HPV prevalence



[a]Data from a representative sample of the US population, from the National Health and Nutrition Examination Survey (NHANES)[22]

[b]Data from the Multicenter AIDS Cohort Study (MACS) and Women's Interagency HIV Study (WIHS),[16] HIV-uninfected individuals in this study were are at-risk for oral HPV due to their higher number of sexual partners and higher use of tobacco

Table 1.1: Summary of results from studies reporting prevalence of oral HPV DNA among HIV-infected adults, contrasted with US general population results

Study	Description	Sample Size	Sample Method	Any HPV*	Oncogenic HPV&	HPV16	Multiple types
Coutlee STD 1997[19]	US men and women	201	oral brush	14%	12%	3.0%	0.5%
Kreimer et al JID 2004[3]	US men and women	190	oral rinse/brush biopsy	25%	14%	---	5.8%
Cameron STD 2005[17]	US men and women	98	Saliva	37%	26%	6.1%	7.0%
Marais JMV 2008 [84]	South African women	33	oral brush	33%	12%	3.0%	9.1%
Richter JOPM 2008[85]	South African women	30	oral brush	20%	7%	0.0%	6.7%
Fakhry Plos One 2010~[86]	US men and women	112	oral rinse	45%	26%	5.9%	---
Parisi BMCID 2011[87]	Italian MSM	166	oral swab	20%	1.5%	0.8%	---
Beachler et al CEBP 2012[16]	US MSM and women	379	oral rinse	40%	21%	6.1%	19%
Read et al Plos One 2012[20]	Australian MSM	249	oral rinse/brush	19%	8%	4.4%	7.2%
Del Mistro STD 2012[88]	Italian men and women	100	Saliva	37%	13%	3.0%	6.0%
Steinau et al JOPM 2012[89]	US men and women	100	oral rinse	39%	24%	3.0%	17%
Fatahzadeh et al OOOOE 2013 [90]	US men and women	52	oral rinse	38%	23%	6.0%	---
Videla et al STD 2013[21]	Spanish men	650	oral brush/rinse	16%	15%	5.2%	3.8%
Beachler et al JID 2013	US men and women	404	oral rinse	28%	13%	2.3%	11%
------	*HIV+ Summary^*	*2764*	-----	*26%^*	*15%^*	*4.2%^*	*8.5%^*

Gillison et al JAMA 2012[22]	*US General Population*[+]	*5501*	*oral rinse*	*7%*	*3.7%*	*1.0%*	---

*Number of total alpha HPV types tested varied from 9 (Coutlee 1997) to 47 (Parisi 2011) although untyped genotypes were included in the prevalence estimates for several studies (Coutlee 1997, Cameron 2005, Del Mistro 2012). Cutaneous HPV types were not included in this summary.
&Number of oncogenic types varied from 7 (Coutlee 1997) to 22 (Kreimer 2004, Marais 2008) while most considered 13-14 types to be oncogenic
~Average point prevalences reported
^Pooled oral HPV prevalence summary includes studies with differences in sample collection, processing, number of HPV types tested, DNA detection methods, and the study participant characteristics
[+]Data from a representative sample of the US population, from the National Health and Nutrition Examination Survey (NHANES)

Table 1.2: Increased risk of head and neck squamous cell carcinoma comparing HIV-infected individuals with the general population

Study	Study Population	Type of Cancer	Standardized Incidence Ratios (SIRs) and (95%CIs)	
			Overall	HIV-Transmission Subgroup#
Shiels et al. JAIDS 2009[4&]	Meta-analysis of developed countries (1980-2007)	Head and Neck	2.0 (1.1-3.6)	---
Simard et al. AIM 2010[27^]	United States (1996-2006)	Oral Cavity and Pharynx	1.8 (1.5-2.0)	---
Silverberg et al. CEBP 2011[29]	United States (1996-2008)	Oral Cavity and Pharynx	aRR*=1.4 (0.9-2.1)	---
Shiels et al. JAIDS 2009[4&]	Meta-analysis of developed countries (1980-2007)	Oropharyngeal	1.9 (1.2-2.5)	---
Chatervedi et al. JNCI 2009[28]	United States (1980-2004)	Oropharyngeal	1.6 (1.2-2.1)	MSM: 1.1 (0.7-1.8), IDU: 2.1 (1.3-3.2), Hetero:3.2 (1.6-5.7)
Clifford et al. JNCI 2005[26]	Switzerland (1985-2002)	Lip, Oral Cavity and Pharynx	4.1 (2.1-7.4)	MSM: 2.0 (0.4-5.8), IDU: 13.7 (4.9-30.1), Hetero: 2.9 (0.3-10.5)
Frisch et al. JNCI 2000[25^]	United States (1987-1996)	Tonsillar	2.6 (1.8-3.8)	Hetero Men: 5.3 (1.1-15.4)

&Meta-analysis included three studies considering oropharynx cancers and four studies exploring head and neck cancer.

^Both studies used data from the US HIV/AIDS Cancer match study

*Relative Risk based on an observational study controlling for potential risk factors such as tobacco and alcohol use

#MSM= Men-who-have-sex-with-men, IDU=Injection drug user, Hetero=Heterosexual

Table 1.3 - Description of studies included in dissertation analyses

	Chapter 2	Chapter 3	Chapter 4
Study Name	Human Oral Papillomavirus Etiology Study (HOPE)	Persistent human Oral Papillomavirus Study (POPS)	North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD)
Description	Prospective cohort study	Prospective study utilizing the MACS and WIHS cohorts	17 Clinical and Interval Based studies
Sample Size	404	1,230	82,375
Years Studied	2006-2009	2009-2013	1996-2010
Locations	Baltimore, MD	Baltimore, MD; Washington, DC; Chicago, IL; Bronx, NY; Brooklyn, NY; Pittsburgh, PA	Various sites in United States and Canada
Risk Groups	HIV-infected Men who have sex with men (MSM), Women, and Heterosexual Males	HIV-infected and HIV-uninfected Men who have sex with men (MSM) and women	HIV-infected: All risk groups

Chapter 2: Natural history of anal versus oral HPV infection in HIV-infected men and women

Natural history of anal versus oral HPV infection in HIV-infected men and women

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ABSTRACT

Background: HIV-infected individuals are at greater risk for HPV-associated anal than oropharyngeal cancers. The prevalence of anal versus oral HPV infections is higher in this population, but whether this is explained by higher incidence or persistence is unknown.

Methods: Oral rinse and anal swab samples were collected semi-annually from 404 HIV-infected adults in Baltimore, MD. Samples were tested for 37 HPV types using PGMY09/11 primers and reverse line-blot hybridization. Risk factors for HPV persistence were explored using adjusted Wei-Lin-Weissfeld models.

Results: The prevalence (84% vs. 28%), incidence (145 vs. 31 per 1000 person-months) and twelve-month persistence (54% vs. 29%) were higher for anal versus oral HPV infections, respectively (each $p<0.001$). Heterosexual men had lower incidence of anal HPV than men-who-have-sex-with-men and women, but a higher incidence of oral HPV infection (p-interaction<0.001). In adjusted analyses, risk factors for HPV persistence included prevalent versus incident (aHR=4.0, 95%CI=3.5-4.8) and anal versus oral HPV infections (aHR=1.5, 95%CI=1.2-1.9).

Conclusion: The higher incidence and persistence of anal versus oral HPV infections likely contributes to the higher burden of anal as compared to oral HPV-associated cancers in HIV infected individuals.

INTRODUCTION

HIV-infected individuals are at elevated risk for all HPV-associated cancers, including cervical, anal and oropharyngeal cancers.[1-3] However, the standardized incidence ratios for these cancers among HIV-infected individuals compared to the general population varies considerably by anatomic site. For example, HIV-infected individuals have over a 25-fold greater risk of anal cancer relative to the general population, but the risk for oropharyngeal cancer is only 2-6 fold greater.[4,5]

The underlying reasons for the different magnitudes of risk for HPV-associated cancers among HIV-infected individuals are unclear. In the case of cervical cancer, the elevated risk among immunosuppressed populations has been attributed to both higher incidence[6,7] and higher persistence rates[6] of cervical HPV infection. Immunosuppression may also increase risk of disease progression.[8,9] Cross sectional studies have consistently shown the prevalence of anal HPV to exceed that of oral HPV among HIV-infected individuals.[10-12] To investigate the possible contribution of differences in incidence and persistence rates to the differing anal-oral HPV prevalence and cancer rates in HIV-infected individuals, a prospective study was performed to compare the natural histories of anal and oral HPV infections among HIV-infected individuals.

METHODS

Study Population and Data Collection

A convenience sample of 404 HIV-infected men and women was recruited from a clinic (the Moore clinic) dedicated to the care of HIV-infected individuals at the Johns

Hopkins Hospital (JHH) in Baltimore, MD in 2006. The study was approved by the Institutional Review Board at JHH, and written informed consent was collected from all participants.

Participants were followed semi-annually for up to two and a half years. Baseline demographic and behavioral information was collected by use of an audio computer assisted self-interview (ACASI) that obtained information regarding demographics, sexual history and drug use. Measurement of current CD4 T cell count (Beckton Dickenson BD TriTest) and HIV RNA viral load (Roche AMPLICOR™ HIV-1 Monitor Test, version 1.5) at baseline was performed unless available in the medical record from within six weeks of that study visit. Nadir CD4 T cell count and current antiretroviral therapy (referred to hereafter as ART, also known as HAART or effective ART) were abstracted from the medical record. ART was defined as the use of three or more antiretroviral medications, which includes a protease inhibitor (PI), a non-nucleoside reverse transcriptase inhibitor (NNRTI) and one of the NRTIs abacavir or tenofovir, an integrase inhibitor, or an entry inhibitor.[13]

Specimen Collection, Processing and DNA Purification

At each visit, exfoliated oral epithelial cells were collected by use of an oral rinse and gargle with 10mL of Scope™ mouthwash.[14] A physician collected anal samples by use of a saline-moistened Dacron swab inserted six cm into the anal canal and removed in a circular motion against the anal wall. The swab was placed in 1 mL of Sample Transport Medium (Digene Diagnostics, Silver Spring, MD).[11]

Strict procedures were used to prevent specimen contamination as described in Koshiol et al.[15], including placement of one negative control per seven experimental samples

in the PCR plate. DNA was isolated from anal swab and oral rinse samples by centrifugation, re-suspension in phosphate buffered saline and purification by use of a modified method for the Puregene DNA purification kit (Gentra Systems, Minneapolis, MN). Purified DNA was evaluated for the presence of 37 HPV types by use of PGYM09/11 PCR primer pools and reverse line blot hybridization (Roche Molecular Systems).[14,16] Samples that were β-globin and HPV-negative were considered inevaluable. Line blots were independently interpreted by two technicians and discrepancies resolved by the principal investigator (MLG).

Statistical Analyses

Associations between baseline characteristics and HPV infection (or number of visits) were evaluated using chi-squared (χ^2) tests for categorical data and t-tests for continuous variables. Baseline and follow-up HPV DNA prevalence and 95% confidence intervals (CIs) were calculated for HPV16, overall HPV (all 37 types), any oncogenic (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and 73) and any non-oncogenic (6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 81, 82, 83, 84, 89 and IS39) type.[17-19] HPV infection status was evaluated both by person and by type-specific infection. In analyses by individual, a participant was considered infected if one or more of the 37 evaluated HPV types were detected at each visit. In analyses by infection, presence of each of the 37 type-specific HPV infections was evaluated for each individual at each visit (point prevalence). Type-specific HPV infections were categorized as prevalent if positive at baseline and incident if first detected thereafter. A type-specific infection was only considered to be incident once, even if variably detected throughout the study (for example: -+-+). Incidence rates were summarized as the number of type-specific infections in the population per 1000 person-

months. Newly detected anal and oral HPV infections were compared with Hazard Ratios (HRs) calculated using the Wei-Lin-Weissfeld (WLW) method,[7,20] which is a marginal model that consists of a Cox proportional hazard model with an exchangeable correlation structure and robust variance to adjust for the correlation between multiple HPV infections detected within a single person.[7,21]

For prevalent and incident infections, the proportion of infections that persisted to 6, 12, 18 and 24 months (since infection) were summarized and compared for anal and oral HPV infections using prevalence ratios (PRs) from log-binomial regression with Generalized Estimating Equations (GEE). Two definitions of type-specific HPV clearance were considered: type-specific detection followed by a single negative test or followed by two consecutive negative tests. All infections defined as persistent required at least two HPV positive visits. Re-detected infections that were initially defined as cleared by either definition were not included in these analyses.

The number of lifetime sexual partners was defined for women as the number of vaginal partners and for men was defined as the sum of the reported number of vaginal and anal sex partners. Men were categorized based on self-reported sexual preference as either heterosexual men preferring only sex with women, or as men who have sex with men (MSM). All women were grouped together, as only ten women reported their sexual preference as lesbian or bi-sexual. Tobacco exposure included use of cigarettes, pipes, cigars, chewing tobacco or snuff, and was described as never, former and current use at baseline. Hard drug use was defined as ever using heroin, cocaine, or crack. Recent or current behaviors were considered as those performed within six months before this study.

Risk factors for prevalent HPV at baseline were explored using log-binomial regression with GEE. Unadjusted and adjusted prevalence ratios (PRs and aPRs) and their 95% confidence intervals (CIs) were reported. Risk factors significantly associated with anal or oral prevalence ($p<0.05$) along with factors shown to be relevant based on previous literature were included in the final adjusted models. The combined model considered both anal and oral HPV infections as outcomes, and an interaction term with each risk factor and the site of infection was evaluated. Trends were evaluated using continuous values for ordinal or continuous risk factors, when available.

Patterns of HPV infection were also described in an analysis restricted to those participants with at least two follow-up visits after their first detection of HPV. HPV patterns for each infection were defined as persistent (if positive for all the visits following the initial positive visit), cleared (if negative for at least the final two visits), or intermittent (if varying between positive and negative throughout the visits).

Time to event outcomes (HPV clearance and persistence) were summarized using Kaplan Meier curves, where infections were either considered cleared at the first negative visit or only after two consecutive negative visits. The inverses for each association were then calculated and reported as HPV persistence. Risk factors for anal and oral HPV persistence were explored using the Wei-Lin-Weissfeld (WLW) method. Unadjusted and adjusted hazard ratios (HR and aHRs) were reported along with their 95% CIs. For the primary persistence analyses, we carried forward the previous visit's HPV results for missing intermittent visits if they were subsequently positive in the next detected visit (example: +.+). All statistical tests were two sided and considered significant at an alpha level=0.05. STATA version 11.0 (Stata Corp) was used for statistical analyses.

RESULTS

Baseline Characteristics

The study population was primarily African American (82%) and the median age was 46 years (Table 2.1). Participants included 153 women (39%), 168 heterosexual men (43%) and 69 men-who-have-sex-with-men (MSM-18%). A majority (76%) reported ever performing oral sex, while approximately a third (36%) had ever had receptive anal sex. Among the 404 individuals enrolled, the median length of follow-up was 18.2 months (IQR=6.2, 24.0), with a maximum of 31.6 months. Characteristics of participants who were lost to follow-up were similar to those who remained for the full duration of the study (six visits). In addition, participants contributing four or more visits were also similar to those with three or fewer visits, with the exception of a higher prevalence of ever use of ART ($p=0.04$) among those with four or more visits.

Anal and Oral HPV prevalence

A total of 1,137 anal swab samples were collected from the 404 individuals over the study period, and 99.1% were evaluable for detection of HPV infection. Prevalent anal HPV infections were common at baseline (84%, 95%CI=80%-88%, Table 2.1), as were multiple concurrent infections (71%, median 4 [IQR=1-7] HPV types per person). Baseline prevalence was highest for anal HPV types 16 (20.3%), 61 (20.0%) and 55 (19.5%). Younger age, sexual orientation, a high number of lifetime anal sex partners and lower current CD4 T cell count were associated with prevalent anal HPV infection (each $p<0.05$, Table 2.2).

There were 1,292 oral rinse samples collected from the 404 individuals throughout the study and 99.1% were evaluable. Prevalent oral HPV infection at baseline was

significantly less common than anal HPV infection (28% vs. 84%, p<0.001, Table 2.1), and 11% of individuals had multiple concurrent oral HPV infections. Baseline prevalence was highest for oral HPV types 55 (4.3%), 83 (4.1%), 72 (3.8%). There were no risk factors independently associated with prevalent oral HPV infection. After adjustment for risk factors such as sexual behavior and current CD4 T cell count, prevalent anal HPV infection was 10-fold more common (aPR= 9.8, 95% CI=7.8-12.2) than prevalent oral HPV infection.

Anal and Oral HPV Incidence

Given the higher prevalence of anal versus oral HPV infections, we compared incidence rates at the two anatomic sites. The incidence of anal infection was significantly higher than for oral infection (145 vs. 31 infections per 1000 person-months, aHR=4.7, 95%CI=3.6-6.2, Table 2.3) particularly among oncogenic types (oncogenic vs. non-oncogenic, p-interaction=0.01). The anal HPV incidence was higher than oral HPV incidence among women (184 vs. 22 per 1000 person-months), MSM (165 vs. 31 per 1000 person-months) and heterosexual men (96 vs. 38 per 1000 person-months, for all p<0.001). Of note, heterosexual men had the highest incidence of oral, but the lowest incidence of anal HPV infections (p-interaction<0.001). Poisson regression with GEE was utilized to compare the incidence rates of anal and oral HPV infection, and the incidence rate ratios between anal and oral HPV were similar to the reported WLW hazard ratios in Table 2.3 (data not shown).

We explored baseline factors associated with incident infections at both anatomic sites (Table 2.2). Heterosexual males were significantly less likely to have incident anal HPV, while significantly more likely to have incident oral HPV (p-interaction<0.001). The incidence of anal HPV infection was similar among individuals who did and did not report

recent anal sex (161 vs. 140 per 1000 person-months, p-trend=0.54), while the incidence of oral HPV was similar among those who did and did not report recent oral sex (36 vs. 27 per 1000 person months, p-trend=0.15).

Anal and Oral HPV persistence

Given the observed higher prevalence and incidence of anal versus oral HPV infections, we also compared persistence of infections at the two anatomic sites. When first considering HPV persistence, we noted that many anal and oral HPV infections had a pattern of intermittent detection during the study (for example: +-+).

To further investigate patterns of infections, we performed an analysis restricted to the 1,426 HPV infections among the 207 individuals with at least two follow-up visits following their first detection of HPV. Individuals in this analysis had a median follow-up of 24.0 months (IQR=18.6-24.5). In this analysis, 38% of all anal and oral infections were persistent (positive for all the visits following the initial positive visit), 26% were intermittent (varied between positive and negative throughout the visits) and 37% were cleared (negative for at least two consecutive visits and not positive for any subsequent visits) (Figure 2.1).

Given these patterns of infection, we considered both a single negative and two consecutive negative definitions for clearance. Regardless of the clearance definition, anal HPV infections were significantly more likely to persist than oral infections (Table 2.4 and Figure 2.2). After six months, 63% of prevalent anal HPV infections persisted while only 46% of prevalent oral HPV infections persisted when using the single negative definition (PR=1.4, 95%CI=1.1-1.7, Table 2.4). Over the course of the entire two and half year study, prevalent anal HPV infections were approximately twice as likely to persist as prevalent oral HPV infections when clearance was defined by a single negative test (HR=1.9, 95%CI=1.6-

2.3, p<0.001, Figure 2.2A) or two consecutive negative tests (HR=2.0, 1.5-2.6, p<0.001, Figure 2.2C). Incident anal infections had a non-significantly higher persistence rate compared to incident oral infections using the single negative test definition (HR=1.3, 95%CI=0.99-2.3, p=0.06, Figure 2.2B), and a modestly higher persistence rate using the two consecutive negative definition of clearance (HR=1.6, 95%CI=1.0-2.4, p=0.03, Figure 2.2D). Results were similar when excluding those individuals with missing intermittent infections.

Factors related to anal and oral HPV persistence

Adjusted WLW models were used to evaluate factors independently associated with anal and oral HPV infection persistence (Table 2.5). Heterosexual males were less likely than MSM and women to have a persistent anal HPV (aHR=0.73, 95%CI=0.57-0.92) but not oral HPV (aHR=1.4, 95%CI=0.91-2.0, p-interaction=0.02). Age, current tobacco use and current and nadir CD4 T cell count at baseline were not associated with anal or oral HPV persistence (Table 5). When anal and oral infections were included in the same model, anal infections were significantly more likely to persist than oral infections (aHR=1.5, 95% CI=1.2-1.9) as were prevalent as compared to incident HPV infections (aHR=4.0, 95% CI=3.5-4.8). Risk factors for persistence were similar among the 207 individuals with at least two follow-up visits following their first detection of HPV and between oncogenic and non-oncogenic HPV types (Table 2.6). In addition, associations between risk factors and patterns of persistent HPV infection (calculated using multinomial logistic regression with GEE) were similar to the WLW analysis except that the anal HPV persistence pattern was even stronger than the pattern of oral HPV persistence (aOR=2.0 (1.1-3.7), Table 2.7).

DISCUSSION

To our knowledge, this is one of the first prospective studies of the natural history of anal and oral HPV infection in HIV-infected individuals. The higher prevalence, incidence and persistence of anal versus oral HPV infections we observed likely contribute to the higher risk of anal as compared to oral HPV-associated cancers in this population.[4,5]

The higher prevalence of anal versus oral HPV infections observed in this study is consistent with prior reports.[10,12,22] Our data indicate that the higher prevalence is due to a combination of a considerably higher incidence rate and a modestly higher persistence rate of anal as compared to oral HPV infections.

These data support a hypothesis of an influence of anatomic site of infection on the natural history of HPV. Possible factors contributing to the observed differences in incidence include variation in the frequency of sexual behaviors related to transmission, local mucosal immunity differences such as the continuous flow of saliva and associated immunoglobulins in the oral region, sample collection differences and a higher propensity for epithelial microtrauma in the anal region.[23] The different methods for oral (rinse) as compared to anal (swab) sampling might affect detection of persistent infection, however research has shown similar rates of HPV type-specific agreement for oral (rinse), anal (swab) and cervical (brush) infections with repeat sampling. [14,16,24]

With regard to sexual behavior, the observed incidence of anal HPV infection was high among MSM, but also among women and heterosexual men. A high anal HPV prevalence among men and women in the absence of receptive anal sex is consistent with other recent studies involving HIV-infected[11] and HIV-uninfected individuals.[25,26] It has been

suggested that non-penetrative sexual behaviors and auto-inoculation through HPV shedding from vaginal discharge could lead to anal HPV infection.[11,26-28] Indeed, HPV type-specific concordance of anal and genital infections has been observed among heterosexual men and women reporting never having anal sex.[11,26-28] Similar to another recent study,[29] we found that anal HPV detected among heterosexual men was less common and less likely to persist than in MSM and women. Notably, the incidence of oral HPV infections was higher among heterosexual men than MSM and women. This is consistent with the higher prevalence of oral HPV infection among men[30] as well as higher rates of HPV-associated oropharyngeal cancer among men in the U.S..[31]

We observed a large proportion of type-specific infections to have an intermittent pattern of detection at both anal and oral sites in this HIV-infected population. Similar patterns have been previously reported for cervical HPV in both HIV-infected[6] and HIV-uninfected[32,33] individuals. It is unclear as to whether intermittent detection is representative of newly acquired infection, reactivation of latent infection or rather limitations in sampling or fluctuation of HPV viral loads around the lower limits of assay detection. We acknowledge that some of the newly detected infections classified as incident likely represent true prevalent infections with intermittent detection. In support of this, incident anal and oral HPV infections were observed among individuals who reported no recent anal or oral sex. In the cervical literature it remains unclear as to whether or not consistent HPV detection versus intermittent HPV detection carries different risks with regard to disease progression. However, HIV-infected women with transient cervical HPV infections have a fivefold increased risk of pre-cancer compared to women without cervical HPV infection.[34]

Similar to previous studies, baseline oral and anal HPV *prevalence* was associated with reduced CD4 T cell count.[11,12] Yet, unlike other anogenital HPV studies,[7,29,35,36] persistence of oral and anal HPV was not associated with severity of immunosuppression. This difference may be explained by our limited sample size or by the fact that the risk factor information such as tobacco use and sexual behavior were only collected at baseline. The lack of time updated measures limits the interferences between HPV persistence and the risk factors of interest as they are prone to unmeasured confounding or time dependent bias.[37,38]

There were several limitations to this study. This population of urban HIV-infected MSM, women and heterosexual men posed several challenges to retention including relocation, incarceration and death that led to non-optimal loss to follow-up and missing intermittent visits. However, those lost to follow-up were similar in baseline characteristics (CD4, drug use) to those who continued to participate throughout the study and results were similar in the multinomial pattern analysis (restricted to those with 4+ visits) compared to the WLW analysis (included all participants). In addition, risk factors were only available at study baseline and we have likely underestimated the true prevalence of oral HPV infection in the study population by restricting our analysis to the 37 HPV types represented on the line blot.[39,40] Participants were recruited through a convenience sample and may not reflect all HIV-infected individuals in this clinic setting, or the larger HIV-infected community. Given the unique aspects of the cohort, including HIV-related immunosuppression and heavy drug use, these results should not be generalized to healthier populations.

The study also had notable strengths. The cohort used well validated statistical and laboratory methods[7,14,16] to explore for one of the first times, the type specific oral HPV natural history, and compare this to anal HPV infection in the same individuals. The study

population included individuals with a wide range of CD4, including severely immunosuppressed individuals, and included MSM, heterosexual men and women.

HIV-infected individuals appear able to clear many anal and oral HPV infections, but persistent and intermittent infections remain common. Higher anal than oropharyngeal cancer rates in HIV-infected individuals may be explained both by higher incidence of infection and higher persistence of anal compared to oral HPV infection. Further research is needed to better understand the natural history of anal and oral HPV infection including the potential impact of screening and the prophylactic HPV vaccines, particularly among these groups at greater risk for HPV-associated cancer.

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Table 2.1 – Baseline characteristics of the 404 participants in this Baltimore, MD based study, 2006-2009

	N	%
Demographics		
Median Age at Baseline [IQR]	46.4 [42.1-51.0]	
Sexual Orientation		
Men who have sex with men (MSM)	69	18%
Heterosexual Men	168	43%
Female	153	39%
Race/Ethnicity		
African American non-Hispanic	317	82%
White non-Hispanic	43	11%
Hispanic, Native American, Asian	21	5.40%
Other	7	1.80%
Education		
< High School Degree	180	46%
High School Degree Only	119	31%
Some college / college graduate	91	23%
Drug and Sexual Behavior		
Tobacco Use		
Never	88	23%
Former	34	9%
Current	268	69%
Hard Drug use (any crack, cocaine, or heroin)		
Never	50	13%
Former	182	47%
Current	158	41%
Lifetime Sexual Behavior		
Ever had receptive anal sex among women	61	40%
Number of vaginal partners among women, median (IQR)	8 [3, 20]	
Number of vaginal partners among heterosexual men, median (IQR)	20 [5, 38]	
Number of anal sex partners among MSM, median(IQR)	13 (3, 38)	
Number of oral sex partners performed on, median (IQR)	4 (1, 9)	
Ever engaged in rimming (oral-anal)	95	26%
Current (Past Six Months) Sexual Behavior		
Recent receptive anal sex among women	18	12%
Recently had vaginal sex among women	78	51%
Recently had vaginal sex among heterosexual men	89	54%
Recent receptive anal sex among MSM	29	42%
Recently performed oral sex (1 or more partners)	137	35%
Always used condoms for vaginal and anal sex	143	40%

HIV-Related Biologic Measures		
Nadir CD4 cell count: median [IQR]	169 [62-286]	
250 or more cells/mL	120	32%
100-250 cells/mL	133	35%
Less Than 100 cells/mL	128	34%
Current CD4 T cell count: median [IQR]	304 [183-502]	
500 or more cells/mL	97	25%
200-500 cells/mL	173	45%
Less than 200 cells/mL	115	30%
HIV RNA viral load: median [IQR]	5583 [400, 41635]	
Less than 400 copies/uL	129	34%
401-20,000 copies/uL	108	29%
20,000 copies/ul or more	141	37%
Ever antiretroviral therapy (ART) use		
No	124	32%
Yes	261	68%
HPV Prevalence at Baseline		
Any HPV (37 types)*		
Anal HPV	315	84%
Oral HPV	108	28%
Oncogenic HPV (14 types)*		
Anal HPV	251	67%
Oral HPV	51	13%
HPV16*		
Anal HPV	77	21%
Oral HPV	9	2.3%
Multiple HPV types*		
Anal HPV	267	71%
Oral HPV	42	11%

*Comparing anal vs. oral, p-value<0.001

Table 2.2 – Risk Factors related to baseline HPV prevalence and incidence by anatomical site

Baseline Characteristics of HOPE participants	Adjusted PR&		Adjusted HR&	
	Prevalent Infection at Baseline		Incident Infection	
	Anal HPV	Oral HPV	Anal HPV	Oral HPV
Age				
Aged 20.7-42.1 (quartile 1)	REF	REF	REF	REF
Aged 42.2-46.4 (quartile 2)	0.84 (0.67-1.1)	0.85 (0.47-1.5)	1.2 (0.84-1.7)	1.6 (0.67-3.7)
Aged 46.5- 51.3(quartile 3)	0.96 (0.78-1.2)	0.92 (0.48-1.8)	0.56 (0.38-0.84)	0.80 (0.40-1.6)
Aged 51.4-60.2 (quartile 4)	0.67 (0.52-0.86)	0.83 (0.44-1.6)	0.58 (0.37-0.90)	0.78 (0.34-1.8)
	p-trend=0.004	p-trend=0.38	p-value<0.001^	p-value=0.07^
Sexual Orientation				
Female	REF	REF	REF	REF
MSM	0.84 (0.64-1.1)	1.1 (0.57-2.0)	0.89 (0.58-1.4)	1.4 (0.53-3.9)
Heterosexual Men	0.47 (0.37-0.59)#	1.3 (0.74-2.2)#	0.64 (0.46-0.88)*	2.1 (1.2-3.9)*
Current Tobacco Smoker				
No	REF	REF	REF	REF
Yes	1.2 (1.0-1.4)	1.2 (0.73-2.0)	0.95 (0.68-1.3)	0.72 (0.40-1.3)
Number of Lifetime Anal Sex Partners				
0 (Never)	REF		REF	
1 to 5	1.2 (1.0-1.5)		1.3 (0.87-1.8)	
6 or more	1.5 (1.1-2.1)		1.4 (0.78-2.4)	
	p-trend=0.17		p-trend=0.27	
Number of Lifetime Oral Sex Partners				
0 (Never)		REF		REF
1 to 5		0.73 (0.39-1.4)		0.48 (0.22-1.0)
6 or more		0.83 (0.38-1.8)		0.94 (0.46-1.9)
		p-trend=0.73		p-trend=0.86
Ever Rimming (performed anal-oral)				
No		REF		REF
Yes		1.1 (0.56-2.2)		0.41 (0.18-0.89)
		p-trend=0.13		p-trend=0.11
Number of recent anal sex partners (receptive)				
0	REF		REF	
1	1.3 (1.0-1.7)		0.67 (0.43-1.0)	
2+	1.2 (0.84-1.6)		1.1 (0.51-2.3)	
	p-trend=0.33		p-trend=0.54	
Number of recent oral sex partners (performed)				
0		REF		REF
1		1.6 (0.86-2.9)		1.5 (0.70-3.2)

2+		1.6 (0.83-3.1) p-trend=0.59		2.1 (0.87-5.2) p-trend=0.15
Condom Use for anal or vaginal sex				
Always	REF		REF	
Never/Sometimes	1.1 (0.88-1.3)		0.91 (0.68-1.2)	
Nadir CD4 T cell count				
>250 cells/mL	REF	REF	REF	REF
100-250 cells/mL	0.89 (0.72-1.1)	0.82 (0.42-1.6)	1.4 (0.94-2.1)	1.7 (0.80-3.4)
<100 cells/mL	0.84 (0.65-1.1)	1.4 (0.71-2.6)	1.0 (0.63-1.5)	2.2 (0.85-5.7)
	p-trend=0.98	p-trend=0.23	p-trend=0.44	p-trend=0.10
Current CD4 T cell count				
>500 cells/mL	REF	REF	REF	REF
200-500 cells/mL	1.4 (1.1-1.7)	1.8 (0.88-3.5)	1.5 (1.1-2.2)	0.69 (0.33-1.5)
≤200 cells/mL	1.6 (1.3-2.1)	1.1 (0.44-2.7)	1.1 (0.71-1.8)	0.49 (0.19-1.3)
	p-trend=0.002	p-trend=0.18	p-trend=0.12	p-trend=0.82

^p-value for age and incident infection compares the two young quartiles to the two older quartiles

*Using a combined prevalent anal and oral model, all tests for interaction between prevalent anal and oral infection were>0.05 except the p-interaction for heterosexual males was <0.001

*Using a combined incident anal and oral model, all tests for interaction between anal and oral incident infection were>0.05 except the p-interaction for heterosexual males was <0.001

&Adjusted modeling only included variables listed in this table

Table 2.3 - Incidence Rates of Oral and Anal HPV Infection per 1000 person-months and adjusted Hazard Ratios (aHR) for anal compared to oral HPV incidence

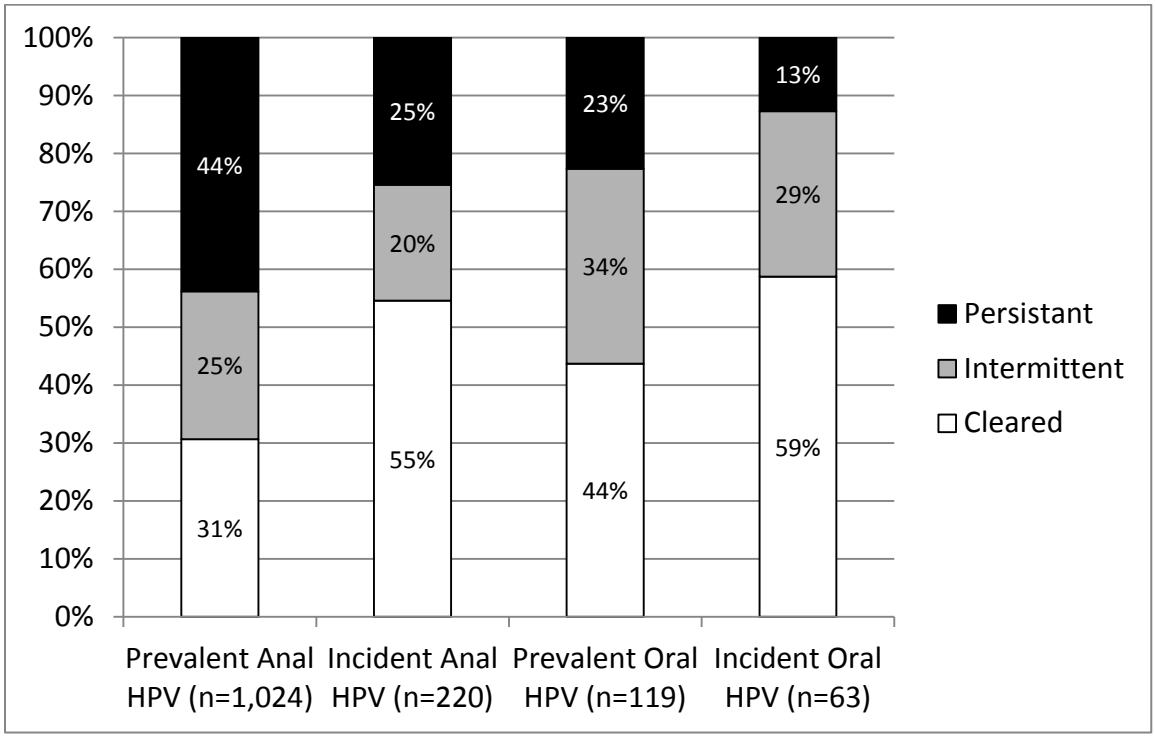
	Incidence Rate (per 1000 person-months)		
	Anal HPV	Oral HPV	Adjusted HR^ (95% CIs)
Overall HPV	145	31	4.7 (3.6-6.2)
Women (n=153)	184	22	7.6 (5.0-11.6)
Men who have sex with men (MSM)(n=69)	165	31	5.7 (2.2-12.4)
Heterosexual Men (n=168)	96	38	2.6# (1.8-3.8)
Oncogenic HPV	60	9.6	6.8 (4.6-10.0)
Non-oncogenic HPV	85	22	3.9* (2.9-5.3)
HPV Vaccine Types (6, 11, 16, 18)	17	4.3	4.9 (2.8-8.5)
HPV16	6.9	1.4	9.7 (3.5-26.8)

^Hazard Ratios calculated using WLW method adjusting for age, gender/sexual preference, tobacco, number of recent sexual (anal/vaginal), number of lifetime sexual partners, nadir and current CD4 T cell count

#p-interaction comparing heterosexual men to MSM and women<0.001

*p-interaction comparing oncogenic and non-oncogenic types=0.01

Figure 2.1 - Patterns of Oral and Anal HPV Infection in the HOPE study^



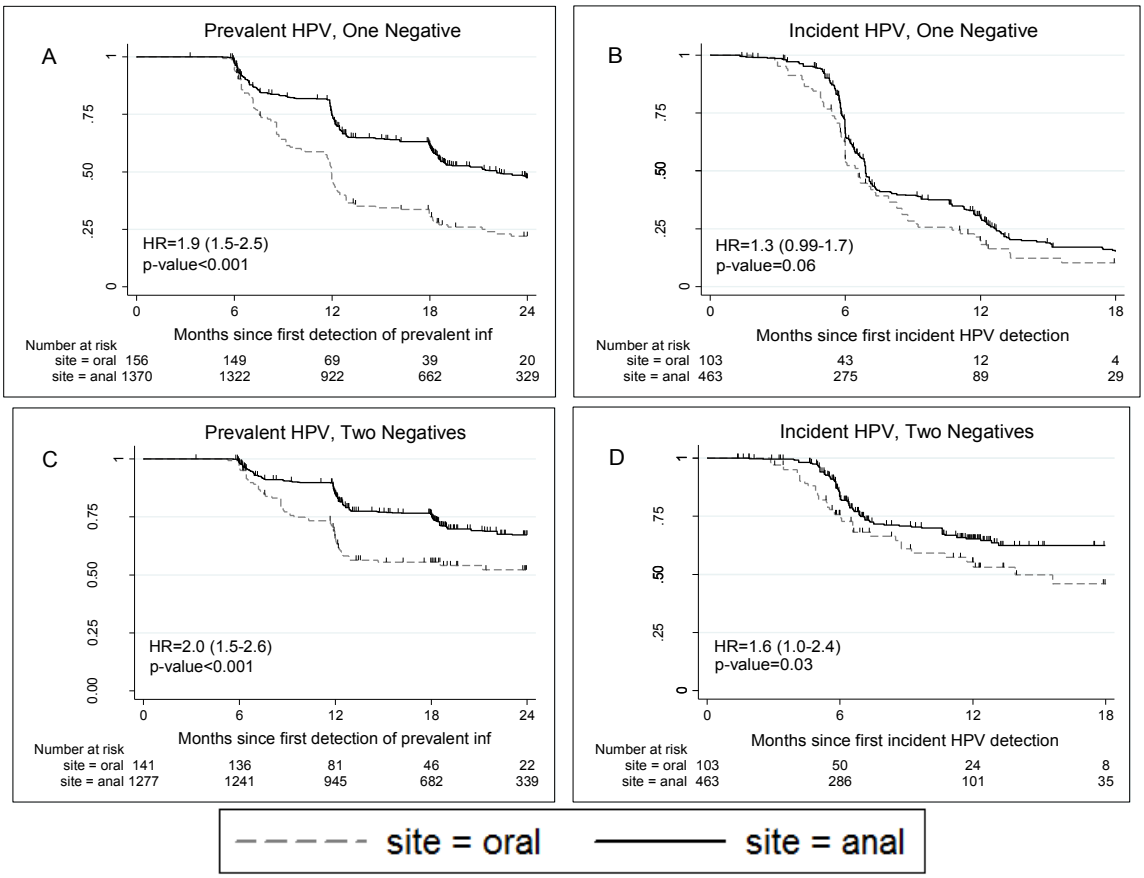
^Restricted to the 207 individuals with at least two follow-up visits following their first detection of HPV

Table 2.4 - Persistence of Anal and Oral HPV Infections, and prevalence ratios (PRs) of anal compared to oral HPV infections

Type of infection and length of persistence	Single negative test for HPV clearance			Two consecutive negative tests required for HPV clearance		
	Any Anal HPV %	Any Oral HPV %	PR (95%CI)*	Any Anal HPV %	Any Oral HPV %	PR (95%CI)*
Prevalent - 6 month persistence	63%	46%	1.4 (1.1-1.7)	77%	63%	1.2 (1.1-1.4)
Prevalent - 12 month persistence	60%	36%	1.6 (1.3-2.1)	73%	53%	1.4 (1.2-1.6)
Prevalent - 18 month persistence	50%	27%	1.8 (1.3-2.5)	65%	48%	1.4 (1.1-1.7)
Prevalent - 24 month persistence	39%	25%	1.5 (1.0-2.3)	58%	47%	1.2 (0.95-1.6)
Incident - 6 month persistence	38%	32%	1.2 (0.82-1.7)	64%	54%	1.2 (0.91-1.5)
Incident - 12 month persistence	28%	15%	1.9 (1.1-3.3)	46%	40%	1.2 (0.78-1.7)
Incident - 18 month persistence	24%	10%	2.4 (0.94-6.2)	36%	28%	1.3 (0.67-2.6)

*Prevalence ratios were calculated using log binomial regression with GEE

Figure 2.2 – Kaplan Meier survival curves comparing the time to clearance of anal vs. oral HPV infections, using a single negative (panel A and B) and two consecutive negative (panel C and D) definitions of clearance*^



*Anal (black) and oral (gray) HPV infections are shown. HPV clearance required only one negative visit in the top two figures, and required two negative visits in the bottom two figures.

^p-values and Hazard Ratios (HR) are from unadjusted WLW model

Table 2.5 – Associations between factors of interest with anal and oral HPV persistence in the HOPE Study

Baseline Characteristics of HOPE participants	Adjusted HR~	
	Persistent Infection&	
	Anal HPV^	Oral HPV^
Age		
Aged 20.7-42.1 (quartile 1)	REF	REF
Aged 42.2-46.4 (quartile 2)	0.96 (0.73-1.3)	1.1 (0.62-1.9)
Aged 46.5- 51.3(quartile 3)	0.82 (0.63-1.1)	1.0 (0.56-1.9)
Aged 51.4-60.2 (quartile 4)	0.86 (0.63-1.2)	0.77 (0.41-1.5)
	p-trend=0.27	p-trend=0.84
Sexual Orientation		
Female	REF	REF
MSM	0.91 (0.72-1.2)	1.2 (0.70-1.9)
Heterosexual Men	0.70 (0.54-0.91)*	1.5 (0.90-2.4)*
Type of Infection		
Incident	REF	REF
Prevalent	4.4 (3.6-5.4)	3.0 (2.1-4.2)
Current Tobacco Smoker		
No	REF	REF
Yes	1.2 (0.92-1.5)	1.0 (0.67-1.6)
Nadir CD4 T cell count		
>250 cells/mL	REF	REF
100-250 cells/mL	1.2 (0.90-1.6)	0.87 (0.55-1.4)
<100 cells/mL	0.76 (1.0-1.4)	1.4 (0.81-2.6)
	p-trend=0.71	p-trend=0.06
Current CD4 T cell count		
>500	REF	REF
200-500	1.0 (0.79-1.4)	1.2 (0.70-2.0)
≤200	1.1 (0.80-1.6)	1.2 (0.59-2.3)
	p-trend=0.77	p-trend=0.09

^Using HPV clearance definition of one negative visit

*Using a combined persistent anal and oral model, all tests for interaction between persistent anal and oral infection were>0.05 except the p-interaction for heterosexual males (vs. women/MSM) was 0.02

~Adjusted analysis included anatomical site, age, sexual orientation, type of infection, tobacco use, nadir and current CD4 T cell count.

&Combined adjusted model found Anal HPV has a 1.5 (1.2-1.9) times higher hazard of persisting than Oral HPV

Table 2.6 - Associations between factors of interest with anal and oral oncogenic HPV persistence in the HOPE study

Baseline Characteristics of HOPE participants	Adjusted HR~ Persistent Onc. Infection&	
	Anal HPV^	Oral HPV^
Age		
Aged 20.7-42.1 (quartile 1)	REF	REF
Aged 42.2-46.4 (quartile 2)	1.0 (0.71-1.3)	0.73 (0.15-3.3)
Aged 46.5- 51.3(quartile 3)	0.91 (0.66-1.3)	1.0 (0.21-5.1)
Aged 51.4-60.2 (quartile 4)	0.84 (0.56-1.2)	0.73 (0.11-5.0)
	p-trend=0.32	p-trend=0.84
Sexual Orientation		
Female	REF	REF
MSM	0.75 (0.54-1.0)	1.5 (0.59-3.9)
Heterosexual Men	0.66 (0.49-0.89)*	1.6 (0.51-5.2)*
Type of Infection		
Incident	REF	REF
Prevalent	4.5 (3.4-5.9)	3.0 (1.3-7.1)
Current Tobacco Smoker		
No	REF	REF
Yes	1.2 (0.88-1.5)	1.4 (0.65-3.1)
Nadir CD4 T cell count		
>250 cells/mL	REF	REF
100-250 cells/mL	1.2 (0.87-1.7)	1.6 (0.54-4.6)
<100 cells/mL	1.0 (0.62-1.6)	1.7 (0.59-4.8)
	p-trend=0.87	p-trend=0.18
Current CD4 T cell count		
>500	REF	REF
200-500	0.93 (0.64-1.3)	1.3 (0.40-4.4)
≤200	1.3 (0.77-2.1)	1.6 (0.43-5.6)
	p-trend=0.94	p-trend=0.64

^Using HPV clearance definition of one negative visit

*In the combined anal and oral HPV model all tests for interaction between persistent anal and oral infection were>0.05, the p-interaction for heterosexual males was 0.06

~Adjusted analysis included age, sexual orientation, type of infection, tobacco use, nadir and current CD4 T cell count.

&Combined adjusted model found Anal HPV has a 1.5 (1.1-2.2) times higher hazard of persisting than Oral HPV

Table 2.7 - Multinomial Regression with GEE to evaluate patterns of infection in the HOPE Study

	Intermittent vs. Cleared Patterns		Persistent vs. Cleared Patterns		Persistent vs. Intermittent Patterns	
	Adj. OR	95% CI	Adj. OR	95% CI	Adj.OR	95% CI
Site of Infection						
Oral HPV Infection	REF		REF		REF	
Anal HPV Infection	0.88	(0.49-1.6)	2.0	(1.1-3.7)	2.3	(1.3-3.9)
Type of Infection						
Incident	REF		REF		REF	
Prevalent	2.57	(1.7-4.0)	2.96	(1.94-4.52)	1.15	(0.79-1.67)
Age						
Aged 20.7-42.1 (quartile 1)	REF		REF		REF	
Aged 42.2-46.4 (quartile 2)	0.55	(0.34-0.88)	0.98	(0.58-1.7)	1.4	(0.84-2.3)
Aged 46.5- 51.3(quartile 3)	0.65	(0.38-1.1)	0.91	(0.52-1.7)	0.95	(0.57-1.6)
Aged 51.4-60.2 (quartile 4)	0.95	(0.57-1.6)	0.9	(0.50-1.6)	1.4	(0.79-2.5)
Sexual Orientation						
Female	REF		REF		REF	
MSM	0.88	(0.57-1.4)	0.96	(0.60-1.5)	1.1	(0.72-1.7)
Heterosexual males	0.73	(0.45-1.2)^	0.56	(0.32-0.99)^	0.77	(0.48-1.3)
Current Tobacco Use						
No	REF		REF		REF	
Yes	1.3	(0.82-1.9)	1.1	(0.70-1.8)	0.90	(0.56-1.4)
Nadir CD4 cell count						
>250 cells/mL	REF		REF		REF	
100-250 cells/mL	1.00	(0.62-1.59)	1.53	(0.82-2.86)	1.54	(0.92-2.58)
<100 cells/mL	0.69	(0.40-1.19)#	1.14	(0.61-2.12)	1.65	(0.95-2.87)#
Current CD4 T cell count						
>500 cells/mL	REF		REF		REF	

200-500 cells/mL	1.38	(0.91-2.1)	1.07	(0.61-1.89)	0.78	(0.48-1.24)
≤200 cells/mL	1.39	(0.76-2.6)	1.36	(0.74-2.51)	0.98	(0.56-1.71)

^p-interaction<0.05. Heterosexual men were more likely to have oral HPV intermittent and persistent patterns than cleared patterns, while they were less likely to have intermittent anal HPV intermittent and persistent patterns than a cleared pattern.
#p-interaction<0.05. Individuals with low nadir CD4 more likely to have intermittent patterns than cleared or persistent patterns for oral HPV but not anal HPV.

Chapter 3: Risk factors for acquisition and clearance of oral HPV infection among HIV-infected and HIV-uninfected adults

Risk factors for acquisition and clearance of oral HPV infection among HIV-infected and HIV-uninfected adults

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Abstract:

Background: Human papillomavirus (HPV) causes the majority of oropharyngeal cancers in the United States, and yet the risk factors and natural history of oral HPV infection are largely unknown.

Methods: Semi-annual oral rinses were collected for up to three years (2010-2013) from 761 HIV-infected and 469 HIV-uninfected participants in the Multicenter AIDS Cohort Study and the Women's Interagency HIV Study. Oral rinses were evaluated for 37 HPV genotypes using the Roche linear array. Factors associated with incidence and clearance were explored using adjusted Wei-Lin-Weissfeld modeling.

Results: The cumulative incidence of type-specific oral HPV infection at two-years was 28%. HIV-infection (aHR=2.3, 95%CI=1.7-3.2) and severity of immunosuppression (current CD4<200: aHR=6.0, 95%CI=2.7-13.3) were associated with increased risk of oral HPV. Additionally, increased risk of infection was associated with younger age, no history of tonsillectomy, and a larger lifetime number oral sex partners in HIV-infected individuals, and with a larger number of recent partners for oral sex, rimming, and performance of oral sex on a woman in HIV-uninfected individuals (all $p<0.05$). Clearance of incident (93%) and prevalent (65%) oral HPV infection within two years was common, nevertheless 23% of all infections persisted to two-years. Older age, current smoking, and male gender significantly reduced clearance rates after adjustment (each $p<0.05$), whereas HIV-infection and severity of immunosuppression did not (p-trend=0.94).

Conclusions: Severity of immunosuppression and sexual behavior increase risk of oral HPV infection whereas male gender, older age and current cigarette smoking increase risk of

persistence. This natural history study thus elucidates the consistent associations observed between these factors and prevalent oral HPV infection.

Introduction:

Oropharyngeal cancers caused by oral human papillomavirus (HPV) infection have increased in incidence over the past several decades, and now represent over half of oropharyngeal cancers in the United States (US).[1,2] Case-control studies report that oral HPV infection greatly increases the odds of oropharyngeal cancer.[3,4] Nevertheless, the natural history of oral HPV has not been well characterized.

Cross-sectional studies estimate that 5-8% of individuals in higher income countries have an oral HPV infection.[5,6] These studies also suggest that prevalent oral HPV infection is more common among men, cigarette smokers, HIV-infected individuals, and those with a higher number of sexual partners.[5,7,8] However, the influence of these factors on the natural history of oral HPV infection is largely unexplored. Preliminary longitudinal studies indicate that oral HPV infection is infrequent and has a high clearance rate.[9,10] Because of a range of factors, including short follow-up duration, a limited sample size, and/or high losses to follow-up,[9-14] the longitudinal studies to date have not adequately identified factors that are independently associated with the acquisition of oral HPV and its subsequent clearance.

For this study, we sought to identify factors independently associated with oral HPV incidence and clearance. HIV-infected individuals are a subgroup of particular interest due to their higher oral HPV prevalence [7,15] and incidence of HPV-related oropharyngeal cancer.[16,17] Therefore, we studied the natural history of oral HPV among a population of HIV-infected and at-risk HIV-uninfected men and women.[7]

Materials and Methods:

Study Population and Study Design:

The Persistent Oral human Papillomavirus Study (POPS) is a prospective study nested within two ongoing longitudinal studies of HIV-infection: the Multicenter AIDS Cohort Study (MACS) and the Women's Interagency HIV Study (WIHS) which have previously been described.[7,18,19] A convenience sample of HIV-infected and at risk HIV-uninfected individuals was enrolled in POPS between October 2009 and March 2011. This study stratified enrollment by study site and HIV-status and individuals who were combination antiretroviral (cART, also known as HAART) naïve were oversampled to allow for a comparison of oral HPV natural history among treated and untreated HIV-infected adults. This study's definition for cART was directed by the US Department of Health & Human Services/Kaiser Panel and is defined as the reported use of three or more antiretroviral medications, which may consist of a protease inhibitor (PI), an non-nucleoside reverse transcriptase inhibitor (NNRTI), one of the nucleoside reverse transcriptase inhibitors (NRTIs) such as abacavir or tenofovir, or an integrase or entry inhibitor.[20]

Men in the MACS (who were primarily men who have sex with men (MSM)) were enrolled from the Chicago, Pittsburgh, and the Baltimore/Washington D.C. sites, whereas women from the WIHS (who were primarily heterosexual) were enrolled from the Chicago, Brooklyn, and Bronx sites. The POPS protocol was approved by the MACS and WIHS executive committees and Institutional Review Boards from each site. All the participants provided written informed consent.

Study participants were followed semi-annually for up to three years (data cut-point: March 2013). At each visit, exfoliated epithelial cells from the oral cavity and pharynx were

collected by use of a 30 second oral rinse and gargle sample collected using Scope™ mouthwash or saline, as previously described.[21,22] Participants underwent a physical examination, venipuncture for CD4 T cell count and HIV RNA viral load, and completed demographic and behavioral surveys.[18,19] At enrollment into POPS, participants also completed a POPS-specific risk factor survey that included additional questions such as lifetime number of oral sex partners. Risk factor data were collected through computer-assisted self-interview (CASI) in the MACS and through interview-administered questionnaires in the WIHS.

The study's definition for the number of oral sex partners included all male or female partners on whom the participant *performed* oral sex on (fellatio or cunnilingus). Rimming was defined as "using your mouth on a partner's anus/butt" (oral-anal contact). Nadir CD4 T cell count was defined as the lowest CD4 T cell count observed in an individual during their participation in the MACS or WIHS or in their abstracted medical record prior to entrance into the MACS or WIHS. Recent behavior was defined as within the past six months.

Laboratory Analysis:

Oral rinse samples were stored at 4º Celsius for up to one week until processed at the Gillison laboratory at the Ohio State University. To avoid specimen contamination, strict laboratory procedures were adhered to and have been previously described.[23] DNA was purified from the oral rinse using a magnetic bead-based automated platform (QIAsymphony SP, Qiagen), as previously described.[24] Purified DNA was then evaluated for 37 different HPV DNA genotypes utilizing PGMY09/11 PCR primer pools followed by reverse line blot hybridization to the Roche™ linear array. Samples were considered evaluable if positive for β-globin, while those negative for β-globin were re-tested and

excluded from the analysis if the re-test was also β-globin negative. Two lab technicians independently interpreted the results; any discrepancies were resolved by the principal investigator of the laboratory (Gillison).

HPV types were classified as either oncogenic (high risk) or non-oncogenic (low risk) utilizing criteria from the World's Health Organization International's Agency for Research on Cancer (IARC).[25-27] HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 73 were classified as oncogenic, while HPV types 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 81, 82, 83, 84, IS39, and CP6108 were classified as non-oncogenic.

Statistical Analyses:

Type specific oral HPV infection was the principal outcome in this study. An infection was classified as prevalent if detected at the baseline visit and classified as incident if detected after a negative type-specific test at baseline. Clearance of type-specific HPV infection was defined in two ways: 1) requiring either a single negative test or 2) requiring two consecutive negative tests. Infections that met either definition were considered cleared at the time of the first negative test.

Participant characteristics by HIV-status and cohort (MACS vs. WIHS) were described and compared utilizing chi-square tests for categorical variables and Mann-Whitney tests for medians for continuous variables. Cumulative incidence curves were utilized to explore the incidence of oral HPV, and Kaplan Meier curves were utilized to determine the time to clearance of oral HPV. The interval between visits in both MACS and WIHS is approximately six months, but the time between visits for participants often varied between five and seven months. Therefore, when reporting oral HPV clearance and cumulative incidence at six, twelve, and 24 months, individuals with visits within one month

of each time point of interest were also included. For infections with missing intermittent visits we used the "carry forward" method, which assumes the HPV results were the same at the missing intermittent visit as the previous visit. Among the 1,270 detected oral HPV infections in this study, 15% of infections had at least one missing intermittent visit, and 4% of infections had two or more missing intermittent visits.

Risk factors were explored using unadjusted and adjusted Wei-Lin-Weissfeld (WLW) models.[28,29] These marginal models utilize Cox proportional hazard modeling, adjust for the correlation between the multiple infections within a single participant, and include an exchangeable correlation structure and robust variance. [28,29] In regression modeling, covariates that were collected semi-annually in MACS and WIHS were utilized as time-varying variables when appropriate (e.g. recent number of oral sex partners, smoking status, and current CD4). Variables only collected once in the baseline survey (e.g. lifetime number of oral sex partners) were included as time-fixed variables in the model.

The initial adjusted WLW models for incidence and clearance of oral HPV infection included variables that were significant ($p<0.05$) in the unadjusted models and those variables considered relevant to oral HPV acquisition and/or clearance based on previous literature. Correlation between covariates was calculated through a Pearson's correlation coefficient. Covariates that were strongly correlated with each other (such as oral sex with rimming and current and nadir CD4 with HIV viral load) were considered in separate models and were evaluated together in the same model when deciding which variables to include in the final model (data not shown). Both the clearance and incidence models considered interactions by HIV-status and gender/cohort.

In a sensitivity analysis to explore intermittently detected HPV type-specific infections, we categorized the infections into discrete patterns as: "persistent" if they were positive at all the visits following an initial positive, "intermittent" if infections varied between negative and positive throughout the study observation, and "cleared" if an infection had at least two subsequent negative visits after a positive and did not re-appear at a later visit.[9] The patterns were then compared using multinomial logistic regression with Generalized Estimating Equations (GEE), and "cleared" infections were used as the reference. All statistical tests were two sided and considered significant using an alpha=0.05 level. All analyses were performed by STATA MP Version 12.0 (STATA Corp, College Station, TX).

Results:

Participant Characteristics:

During this three year study with semi-annual follow-up, 6,065 oral rinse samples were collected from the 1,230 participants who contributed two or more study visits (median=5, interquartile range [IQR]=3-6). There were 24 (0.4%) β-globin negative oral rinse samples excluded from the analysis. Median follow-up time was 24.4 months (IQR=18.4-35.4), and 89% of eligible participants contributed results at the most recent visit.

The characteristics of the 1,230 participants at enrollment are shown in Table 3.1, stratified by cohort (gender) and HIV-status. Among these participants, 45% (n=550) were men from the MACS, and 55% (n=680) were women from the WIHS. The vast majority of

participants were MSM or heterosexual women. Thus, most participants who reported oral sex behavior performed the act on a man (fellatio). However, ~5% of both the MACS and WIHS participants reported having recently performed oral sex on a woman (cunnilingus). When compared to the women from the WIHS, the men from the MACS were older (median: 53.7 vs. 46.4 years) and were more likely to be Caucasian, never smokers (41% vs. 23%), current alcohol drinkers, and more likely to have ever had a tonsillectomy and have a greater number of oral sex and rimming partners (each $p<0.01$, Table 3.1). HIV-infected men were significantly more likely than HIV-infected women to be currently using cART (87% vs. 74%), to have a higher current CD4 T cell count (median: 575 vs. 520 cells/μL), a lower nadir CD4, and a lower HIV RNA viral load (each $p<0.01$, Table 3.1).

This study population includes 469 (38%) at-risk HIV-uninfected, 702 (57%) cART-experienced HIV-infected and 59 (4.8%) cART-naïve HIV-infected individuals. While HIV-infected and HIV-uninfected participants had many similarities, there were some differences in participant characteristics by HIV-status (Table 3.1). HIV-infected individuals were more likely than HIV-uninfected participants to be black, not currently drink alcohol, and to have a lower number of recent oral sex partners (each $p<0.05$, Table 3.1).

Incidence of oral HPV

Incident, type specific oral HPV infections were commonly observed in the study population. The incidence rate of any oral HPV infection was 18.7 infections (95%CI=17.2-20.3) per 1000 person-months, while the incidence of any oncogenic oral HPV and HPV16 were 7.3 (95%CI=6.4-8.3) and 1.0 (95%CI=0.72-1.5) per 1000 person-months, respectively. After 24 months of follow-up, the cumulative incidence of any type-specific oral HPV,

oncogenic oral HPV, and HPV16 were 28% (95%CI=25-31%), 15% (95%CI=12-17%), and 3.5% (95%CI=2.4-5.0%), respectively.

Risk factors for incident oral HPV

Oral HPV incidence was similar between men and women (MACS vs. WIHS: IR=19.5 vs. 17.9 per 1000 person-months, p=0.75), but was significantly higher among HIV-infected than HIV-uninfected individuals (IR=24.1 vs. 10.3 per 1000 person months, p<0.001, Figure 3.1). Further, risk of incident oral HPV infection increased as CD4 T cell count declined (p-trend<0.001, Figure 3.1). Additional factors associated with risk of incident oral HPV infection in unadjusted analysis included never having had a tonsillectomy, younger age, several measures of sexual behavior and HIV-related immunosuppression, such as low nadir CD4 T cell count and high HIV RNA viral load (Table 3.2-4, each p<0.05). In contrast, gender, current cigarette smoking, marijuana use, current cART use, and not always using a condom/dental dam for recent oral sex acts were not associated with higher oral HPV incidence (Tables 3.2 & 3.3, each p>0.05).

After adjustment for demographic and behavioral risk factors, HIV-infection and low current CD4 T cell count were strong risk factors for incident oral HPV infection in both the MACS and WIHS (Tables 3.3, 3.5 and Table 3.10, p-trend<0.001). Individuals with a current CD4 less than 200 cells/μL had a six-fold higher risk (aHR=6.0, 95%CI=2.7-13.3) of incident oral HPV infection than HIV-negative individuals. Current CD4 T cell count was associated with risk of incident oral HPV (p-trend=0.01), even after adjusting for correlated measures, such as nadir CD4 and HIV viral load. In contrast, low nadir CD4 and a higher HIV viral load were not independently associated with risk of incident oral HPV (both p-trends>0.10).

Number of oral sexual partners was an important determinant of the risk of incident infection for both HIV-infected and HIV-uninfected individuals (Table 3.4). Risk of incident oral HPV infection increased with higher number of recent partners for oral sex or rimming and having recently performed oral sex on a woman among HIV-uninfected individuals, but not in HIV-infected individuals (Table 3.4, all p-interactions$\leq$0.05). In contrast, risk increased with higher number of lifetime oral sex partners among HIV-infected individuals, but not HIV-uninfected individuals (although this interaction was not significant, Table 3.4, p-interaction=0.76). Number of oral sex partners was strongly correlated with number of anal or vaginal sexual partners, and total number of sexual partners for any act (r=0.82). Nevertheless, number of recent and lifetime *oral* sex partners remained significantly associated with incident oral HPV risk after adjustment for number of recent and lifetime sexual partners for any act (both p-trends<0.01, Table 3.6).

In addition to immunosuppression and increased number of oral sex partners, risk of incident oral HPV remained significantly elevated among younger participants and in those never having had a tonsillectomy after adjustment (Table 3.3). All of these associations with incident oral HPV infection remained similar when stratifying by gender, with the exception that heavy alcohol drinking increased risk of oral HPV infection in the WIHS but reduced risk in the MACS (p-interaction<0.001, Table 3.5). Additionally, risk factors for oncogenic oral HPV and oral HPV16 incidence were similar to the risk factors for any incident oral HPV infection (Table 3.8, all p-interactions>0.05).

The association between number of lifetime oral sex partners and risk of incident oral HPV infection lead us to investigate the possibility of oral HPV re-activation. To do so, we stratified participants into whether or not they reported any type of sexual activity over

the course of the whole study. The incidence of oral HPV infection among the sexually abstinent participants (n=231, 19% of all participants) was only modestly reduced when compared to the sexually active group (15.6 vs. 19.4 per 1000 person-months, p=0.26). In the sexually abstinent group, HIV-infection, low current CD4, and a higher number of lifetime oral sex partners were associated with increased risk of incident oral HPV (data not shown).

An additional subgroup analysis was performed among the 8% (n=58) of HIV-infected individuals who remained cART naïve throughout the study. Results were similar, although the risk of oral HPV infection associated with increasing number of lifetime oral sex partners was significantly stronger in cART naïve individuals. (≥100 lifetime partners: $aHR_{cART\ naive}$=15.1, 95%CI=2.8-81.6 vs. $aHR_{cART\ experienced}$=2.7,95%CI=1.2-6.0; p-interaction=0.01).

Clearance of oral HPV

The majority of incident oral HPV infections cleared within two-years of follow-up. When oral HPV clearance was defined by a single negative HPV test, 64% (95%CI=60-69%) of incident oral HPV infections cleared within six months, and 83% (95%CI=78%-86%) cleared within a year (Figure 3.2A). One-year clearance rates were similarly high for incident oncogenic HPV (81%), HPV16 (83%) and non-oncogenic HPV (83%) types. In contrast, only half of prevalent oral HPV infections cleared by one-year (51%, 95%CI=47%-55%, Figure 3.2B) and these infections were less likely to clear than incident infections (HR=2.9, 95%CI=2.4-3.6). Of note, 23% of all infections persisted for at least two-years (incident=7%, prevalent=35%).

There were also a considerable number of intermittent infections detected in this study, particularly among HIV-infected individuals. Among incident infections with at least three follow-up visits, 26% were intermittently detected throughout the study (HIV+: 29%, HIV-: 18%, p=0.09), while 11% were persistently detected and 63% cleared. When clearance was defined by two negative HPV tests, overall oral HPV clearance rates were appreciably lower than when a single negative was used to define clearance (one-year clearance: incident: 53% vs. 83%, prevalent: 35% vs. 51%; Figures 3.2C and 2D).

Risk factors for oral HPV clearance

Men were significantly less likely to clear an incident oral HPV infection when compared to women in unadjusted analysis (HR). (One-year clearance rates were 59% vs. 70%, p=0.002), respectively. Additional factors associated with reduced oral HPV clearance in unadjusted analysis including current cigarette use, prevalent oral HPV infection, and older age (each $p<0.05$; Tables 3.2 & Table 3.7). Factors not associated with oral HPV clearance in unadjusted analysis included HIV status, current and nadir CD4 T cell count, current HIV RNA viral load, marijuana use, cART use, alcohol use, and education (all $p>0.05$, Tables 3.2 & 3.7).

After adjustment for other factors, older age, male gender and current cigarette smoking all remained significantly associated with reduced oral HPV clearance (each $p<0.05$, Table 3.7 & 3.10). In addition, incident infections were significantly more likely to clear than prevalent infections (aHR=2.4, 95%CI=2.0-2.8, Tables 3.7 & 3.10). In contrast, HIV-infection and low current CD4 T cell count remained unassociated with oral HPV clearance after adjustment for other risk factors (Table 3.7, p-trend=0.94). Indeed, the lack of

association of HIV and current CD4 with oral HPV clearance contrasts with the strong association of these factors with oral HPV incidence (Table 3.10).

In subgroup analyses, risk factor results for oral HPV clearance were similar when stratifying by HIV-status (Table 3.7) and between cART naïve and cART experienced individuals (all p-interactions>0.05, data not shown). Most of the risk factors for clearance were similar between men and women except that reduced oral HPV clearance was associated with ever smoking cigarettes in the WIHS only and with lower current CD4 in the MACS only (both p-interactions<0.10, Table 3.9). Clearance of oncogenic oral HPV and HPV16 were similar to the risk factors for clearance of any oral HPV infection, except older age was more strongly associated with reduced clearance of oral HPV16 infection (p-interaction=0.02, Table 3.8).

When using a two negative test definition for oral HPV clearance, risk factors for clearance were also similar to those observed with a single negative definition (Table 3.10), except that HIV-infected individuals were significantly less likely to clear oral HPV than HIV-uninfected individuals when using this stricter definition (aHR=0.75, 95%CI=0.60-0.95). Indeed, in a subsequent analysis where infections were categorized as longitudinal patterns, HIV-infected individuals were more likely than HIV-uninfected individuals to have an "intermittent pattern" of oral HPV (aOR=1.6, 95%CI=1.0-2.4), but similarly likely to have a "persistent pattern" (p=0.18). The associations between the other risk factors and patterns of infections, calculated with multinomial logistic regression with GEE, were similar to the results calculated with the main WLW modeling, including current CD4 (data not shown).

Discussion:

In this natural history study of oral HPV infection, HIV-infection, severity of immunosuppression and sexual behavior significantly increased risk of oral HPV infection. In contrast, male gender, older age and current cigarette smoking increased risk of oral HPV infection persistence. Our data therefore explain in part the consistent associations observed between these factors and oral HPV infection in previous cross-sectional studies. [5,7,15,16,30,31]

The majority of incident oral HPV infections, whether oncogenic or non-oncogenic, cleared within two years of follow-up. Only 7% of these incident infections persisted to two years. The natural history of oral HPV infections is thus quite similar to anogenital HPV infections,[32-34] where a similar minority of incident infections (~10%) persist to two years. The higher persistence rates of prevalent versus incident infections have also been observed in the anogenital tract. While anogenital infection persistence is an accepted surrogate for subsequent risk of cervical and anal dysplasia, it remains to be determined whether or not persistent oral HPV infection could be used to identify those at risk for oropharyngeal cancer.

Surprisingly, our study finds that the higher prevalence of oral HPV infection previously observed among immunosuppressed HIV-infected individuals, [7,15,30] is likely explained more by an increased incidence of oral HPV rather than a reduced clearance. This suggests that immunosuppression may act primarily on the earliest stage of the oral HPV carcinogenesis process. The lack of a major impact of immunosuppression on oral HPV clearance is counter to most previous studies of cervical HPV clearance,[35,36] but more consistent with a previous oral HPV[9] and a cervical HPV study in the WIHS.[33] The lack of association with persistence may explain in part the modest increased rate of oropharyngeal

cancer in HIV-infected individuals (SIR=1.5-4).[16,37] We note, however, that this population was relatively immunocompetent and that a modest effect of low CD4 on oral HPV clearance among men was observed in our study. Thus, we cannot exclude a possible effect of more severe immunosuppression on oral HPV clearance.

This is one of the first studies to report that performing oral sex and rimming are both associated with increased risk of oral HPV acquisition. This differs from a recent study among middle aged HIV-uninfected men that reported elevated risk of oral HPV infection among single individuals, but not with recent performance of oral sex.[10] However, single marital status may be a surrogate for new or multiple oral sex partners, as a limitation of both studies is the absence of data collection regarding new versus established partnerships. While oral sex and rimming are suggested transmitters of oral HPV infection in this study, these behaviors are strongly correlated with other sexual behaviors, and we cannot exclude the possibility that oral HPV is transmitted in other ways such through autoinoculation of anogenital HPV infections.[11]

In addition to the association with oral sexual activity, this study suggests that some newly detected (incident) oral HPV infections may be re-activated latent infection, similar to what has been observed in studies of cervical HPV infection.[33,38,39] Along with the quarter of infections that were intermittently detected throughout this study, we observed a substantial number of incident infections among individuals who self-reported not having any form of sex (oral, anal or vaginal) during the study period. In these sexually abstinent individuals, there was a strong association of oral HPV incidence with both immunosuppression and a higher number of lifetime oral sex partners. Thus, the increased risk of incident oral HPV infection among immunosuppressed individuals may be explained by re-activation or re-

acquisition of previous infection due to poor immune surveillance.[40] However, the increased risk of oral HPV among HIV-infected individuals may be due in part to increased establishment of oral HPV upon exposure. A recent study reported that HIV proteins tat and gp120 disrupt the tight junctions in oral and anogenital mucosal epithelium, a factor which may facilitate infection.[41]

In addition to associations with immunosuppression and sexual behavior, younger age and never having a tonsillectomy increased risk of oral HPV infection. To our knowledge, this is the first study to find that individuals with a history of tonsillectomy may have a reduced incidence of oral HPV infection. However, oral HPV prevalence was similar among those with and without a history of tonsillectomy, perhaps due to a non-significantly higher persistence of oral HPV in those with a history of tonsillectomy.

Several factors were associated with reduced clearance of oral HPV infection, including cigarette use, older age and male gender. Higher persistence rates among men are of particular interest given both the higher oral HPV prevalence [5,30] and incidence rates for HPV-positive oropharyngeal cancer among men.[42] Reduced oral HPV clearance among men is consistent with more vigorous immune response to various infectious agents among women when compared to men, potentially due to hormonal differences.[43] In addition, the higher risk of oral HPV infection we observe among individuals reporting recent oral sex on a woman (cunnilingus) (even after adjustment for number of recent and lifetime oral sex partners) is consistent with a hypothesis of higher rates of transmission with cunnilingus than fellatio. [9,16] This could also contribute to higher oral HPV prevalence and oropharyngeal cancer incidence among men. However, as this study included almost exclusively MSM and heterosexual women, we are limited in ability to explore these hypotheses further.

Our results also suggest that the higher prevalence of oral HPV among cigarette smokers,[5,7,31] may be due to a reduced ability to clear oral HPV rather than an increased likelihood to acquire/re-activate oral HPV. Our association between reduced clearance of oral HPV and cigarette smoking was only observed among women, which is consistent with an oral HPV prevalence study in the National Health and Nutrition Examination Survey (NHANES).[5] In addition, our study may help explain oral HPV's bimodal distribution with age, [5] as we suggest that older individuals may have a lower incidence of oral HPV but also a reduced clearance.

Risk factors associated with oral HPV incidence and clearance were similar for non-oncogenic HPV, oncogenic HPV, and for HPV16 infection alone (which causes more than 85% of HPV-associated oropharyngeal cancers).[44] As over 80% of oral HPV16 infections cleared within twelve months in both HIV-infected and HIV-uninfected individuals, a single detection of HPV16 would likely have very low specificity for oropharyngeal cancer as it does for cervical intraepithelial neoplasia in higher risk groups.

This study has several important strengths. It is one of the first large and long-term longitudinal natural history studies that provides detailed information on the clearance of incident oral HPV infections with high follow-up rates and detailed information on risk factors. Previous longitudinal studies have had either little follow-up time, and/or a limited number of participants and infections to follow.[9-14] In addition, our study population is valuable for gaining a better understanding of the natural history of oral HPV given that oropharyngeal cancers often occur at middle age and HIV-infected individuals are at increased risk for this cancer.[16]

However, there are also limitations to this study. First, this study population is higher risk and has reduced generalizability to other populations. Second, the exact time to clearance for oral HPV should be interpreted with caution as our six month sampling interval may have underestimated the incidence and clearance rates considering the transient nature of oral HPV. On the other hand, type-specific infections were commonly re-detected throughout our study so it is also quite possible that using a single negative test *overestimates* the actual clearance rate. As the HPV viral load of some oral infections may have been just below the level of detection of the assay, we likely classified some persistent infections as cleared when using this single negative test definition. This limitation is true for other HPV studies as well, considering oral rinses have shown similar short term percent agreement to anogenital sampling.[21,24,45] Third, we had some missing data including 15% of infections having one missing intermittent visit. However, risk factors for persistence were similar when analyzed as multinomial patterns. Finally, we should note that the oral rinses collect cells from the oral cavity and pharynx and while they have been shown to have a high sensitivity,[30] they may have a reduced specificity since it may also identify infections that may be less relevant to oropharyngeal cancers.

This longitudinal study suggests that the risk of incident oral HPV infection is increased by sexual behaviors and severity of immunosuppression, while the risk of oral HPV persistence is increased by older age, male gender, and current cigarette smoking. We find that oral HPV infections are often transient in both HIV-infected and HIV-uninfected individuals, but a subset of these infections persist for over two years. The risk that these long-term persistent oral HPV infections progress to oropharyngeal cancer is currently unclear. Additional research will be necessary to better understand these long-term persistent

infections as well as the biological underpinnings of associations with gender, age and smoking.

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Table 3.1 – Baseline characteristics by Cohort and HIV-status among 1,230 POPS participants, 2010-2013

Baseline Characteristics	Overall (n=1230)	MACS (n=550)	WIHS (n=680)	HIV-infected (n=761)	HIV-uninfected (n=469)
Age (Median, IQR)	49.7 (43.0-56.2)	53.7* (48.3-60.0)	46.4* (40.1-52.4)	49.4 (43.5-55.2)	50.4 (42.2-58.4)
Race/Ethnicity					
White Non-Hispanic	366 (30%)	62%*	3.8%*	26%&	35%&
Black Non-Hispanic	682 (55%)	32%	74%	59%	51%
Hispanic any race	157 (13%)	4.6%	19%	13%	13%
Other race	24 (2.0%)	1.3%	2.5%	2.2%	1.5%
Study Site					
Baltimore/Washington DC	189 (15%)	34%	--	14%	17%
Pittsburgh, PA	172 (14%)	31%	--	13%	15%
Chicago, IL	398 (32%)	34%	31%	35%	28%
Bronx, NY	206 (17%)	--	30%	16%	18%
Brooklyn, NY	265 (22%)	--	39%	22%	22%
Cigarette smoking					
Never	381 (31%)	41%*	23%*	31%	32%
Former	363 (30%)	31%	29%	29%	30%
Current	476 (39%)	28%	48%	40%	37%
Current alcohol use					
None	658 (54%)	41%*	64%*	59%&	46%&
Less than 2 drinks per day	468 (38%)	46%	32%	35%	43%
2 or more drinks per day	97 (8%)	13%	3.8%	6.2%	11%
Lifetime number of oral sex partners (Median, IQR)	10 (3-61)	61 (30-224)*	3 (1-10)*	10 (3-61)	10 (3-61)
0-4	463 (39%)	13%	59%	40%	39%
5 to 99	462 (39%)	40%	38%	38%	40%
100 or more	266 (22%)	47%	2.8%	22%	21%
Number of recent^ oral sex partners	0 (0-1)	1 (0-3)	0 (0-1)	0 (0-1)	1 (0-2)
0	685 (56%)	35%*	73%*	61%&	49%&
1	283 (23%)	26%	21%	21%	26%
2 to 5	169 (14%)	25%	5.4%	11%	18%
6 or more	77 (6.3%)	14%	0.2%	6.1%	6.7%
Recent^ oral sex on a woman					
No	1,173 (95%)	94%	95%	97%	93%
Yes	62 (5.0%)	5.6%	4.9%	3.3%	6.8%
Number of recent^ rimming partners					
None	755 (81%)	70%*	93%*	81%	80%
One	101 (11%)	15%	5.9%	10%	13%

Two or more	78 (8.4%)	14%	1.6%	9.0%	7.5%
Received HPV Vaccine by baseline visit					
No	1083 (91.2%)	90%	92%	89%	95%
Yes	27 (2.3%)	1.9%	2.5%	2.8%	1.4%
Unsure	77 9 (6.5%)	8.1%	5.2%	8.0%	4.1%
Ever had a tonsillectomy					
No	891 (75%)	59%*	87%*	75%	75%
Yes	278 (23%)	39%	11%	23%	24%
Unsure	19 (1.6%)	2.1%	1.1%	1.8%	1.1%
Recent frequency of toothbrushing					
2 or more times per day	707 (59%)	56%	62%	57%	63%
1 time per day	368 (31%)	35%	28%	31%	29%
<1 time per day	56 (4.7%)	6.1%	3.6%	5.3%	3.6%
No teeth/dentures	65 (5.4%)	3.4%	7.0%	5.7%	5.1%
cART use					
Current	604 (79%)	87%*	74%*	79%	---
Former	98 (13%)	8.6%	16%	13%	---
Never (cART naïve)	58 (7.6%)	4.6%	10%	7.6%	---
Current CD4 T cell count	549 (351-759)*	575^* (389-759)	520^* (316-752)	549& (351-759)	922& (751-1192)
500 or more cells/μL	424 (56%)	60%	53%	56%	96%*
200-499 cells/μL	262 (35%)	34%	35%	35%	4.1%*
less than 200 cells/μL	72 (9.5%)	5.5%	12%	10%	0%*
Nadir CD4 T cell count	292 (185-422)	275^* (179-421)	312^* (188-422)	292& (185-422)	590& (483-757)
500 or more cells/μL	130 (17%)	17%	18%	17%	73%
200-499 cells/μL	409 (55%)	55%	54%	55%	26%
Less than 200 cells/μL	210 (28%)	28%	28%	28%	0.9%
HIV RNA viral load	48 (40-483)*	40 (40-51)#*	48 (48-1513)#*	48 (40-483)	---
Less than 50 copies/mL	485 (64%)	75%	57%	64%	---
50-20,000 copies/mL	192 (25%)	18%	31%	25%	---
20,000 or more copies/mL	77 (10%)	7.7%	12%	10%	---
Number of visits contributed					
2 to 3	221 (18%)	17%	18%	18%	17%
4 to 5	555 (44%)	39%	49%	44%	46%
6 to 7	476 (38%)	43%	33%	39%	37%

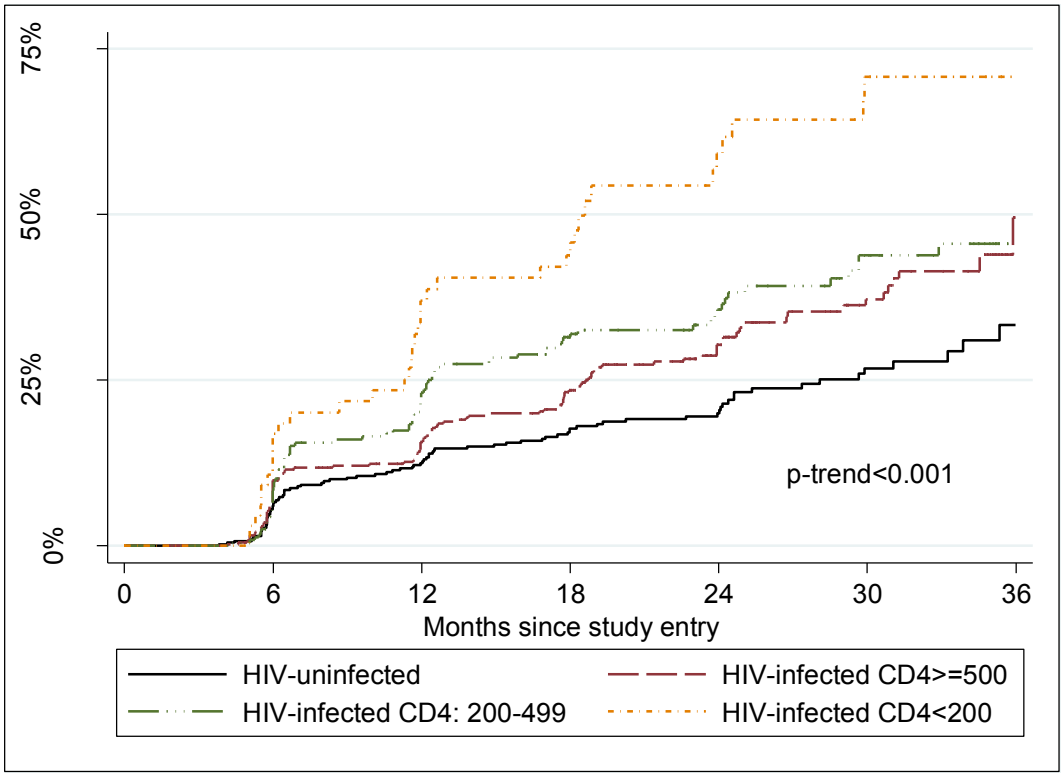
*Factor significantly different between MACS and WIHS participants ($p<0.05$)

&Factor significantly different between HIV-infected and HIV-uninfected participants ($p<0.05$)

^Recent was defined as behavior in the past six month

#Restricting to HIV-infected individuals

Figure 3.1 – Cumulative Incidence of Any Oral HPV Infection by HIV status and CD4 T cell count in the POPS



*p-trend is testing oral HPV incidence by HIV-status and CD4 T cell count using unadjusted Wei-Lin Weissfeld modeling

Table 3.2: Unadjusted associations between risk factors and oral HPV clearance and incidence by gender in the POPS

Characteristics of POPS participants	Unadjusted HR~ (95%CI)			
	MACS Clearance	WIHS Clearance	MACS Incidence	WIHS Incidence
HIV-status				
Negative	REF	REF	REF	REF
Positive	0.90 (0.71-1.1)	0.95 (0.69-1.3)	2.1 (1.5-3.0)	2.7 (1.6-4.5)
Age				
Younger than 45	REF	REF	REF	REF
45-55	0.93 (0.72-1.2)	0.67 (0.46-0.98)	0.71 (0.41-1.2)	0.91 (0.51-1.6)
55 or older	0.72 (0.54-0.96)	0.57 (0.38-0.85)	0.54 (0.30-0.95)	0.73 (0.38-1.4)
p-trend	0.02	<0.001	0.02	0.68
Race/Ethnicity				
White Non-Hispanic	REF	REF	REF	REF
Black Non-Hispanic	1.2 (0.97-1.6)	1.3 (0.88-1.8)	1.8 (1.2-2.6)	1.1 (0.62-2.2)
Hispanic any race	0.85 (0.41-1.8)	1.2 (0.79-1.8)	1.5 (0.60-3.7)	0.45 (0.23-0.89)
Other race	1.8 (1.3-2.4)	2.4 (1.0-5.5)	0.94 (0.30-3.0)	0.50 (0.16-1.5)
Education				
Less than high school degree	REF	REF	REF	REF
High school degree only	REF	1.1 (0.90-1.5)	1.3 (0.56-3.1)	0.70 (0.47-1.0)
Some college or college degree	0.95 (0.77-1.2)	1.3 (0.82-2.1)	0.79 (0.31-2.0)	1.3 (0.66-2.5)
More than a college degree	0.92 (0.68-1.2)	---	0.63 (0.24-1.6)	---
p-trend	0.39	0.23	0.02*	0.54
p-interaction	0.27		0.18	
Cigarette smoking				
Never	REF	REF	REF	REF
Former	0.89 (0.68-1.2)	0.38 (0.18-0.80)	0.77 (0.54-1.1)	1.1 (0.31-4.0)
Current	0.94 (0.72-1.3)	0.36 (0.17-0.77)	0.98 (0.66-1.4)	1.6 (0.45-5.5)
p-interaction	0.03		0.48	
Current cigarette dose				
None	REF	REF	REF	REF
0.1 to 0.49 packs/day	1.0 (0.74-1.4)	0.70 (0.48-1.0)	1.1 (0.64-1.7)	1.5 (0.82-2.7)
0.5-0.99 packs/day	1.1 (0.78-1.4)	0.80 (0.53-1.2)	1.2 (0.80-1.9)	1.5 (0.81-2.9)
1 or more packs/day	1.1 (0.79-1.5)	0.73 (0.46-1.2)	1.4 (0.75-2.6)	1.5 (0.74-3.1)
p-trend	0.59	0.22	0.23	0.14

Current alcohol use				
No	REF	REF	REF	REF
Yes (less than 2 drinks per day)	1.0 (0.85-1.3)	1.0 (0.82-1.2)	0.75 (0.56-1.0)	1.2 (0.89-1.5)
Yes (2 or more drinks per day)	1.1 (0.76-1.7)	1.7 (0.72-3.9)	0.46 (0.26-0.79)	3.1 (1.2-8.1)
p-trend	0.53	0.40	0.02	0.53
p-interaction	0.36		0.001	
Current hard drug use				
No	REF	REF	REF	REF
Yes	1.0 (0.78-1.4)	0.83 (0.60-1.2)	1.3 (0.88-1.8)	1.7 (1.1-2.8)
Current Marijuana Use				
No	REF	REF	REF	REF
Yes	0.93 (0.74-1.2)	0.95 (0.72-1.2)	0.92 (0.68-1.3)	1.1 (0.73-1.8)
Recent oral sex partners				
0	----	----	REF	REF
1	----	----	0.64 (0.44-0.95)	0.93 (0.72-1.2)
2 to 5	----	----	1.1 (0.77-1.6)	1.7 (0.97-2.9)
6 or more	----	----	1.4 (0.91-2.1)	----
p-trend	----	----	0.004	0.97
p-interaction	----	----	0.39	
Recent oral sex on a woman				
No			REF	REF
Yes			1.5 (0.84-2.7)	1.8 (0.83-3.9)
Lifetime oral sex partners				
0 to 4	----	----	REF	REF
5 to 99	----	----	1.0 (0.54-1.9)	2.1 (1.3-3.5)
100 or more	----	----	1.4 (0.84-2.4)	3.0 (1.3-7.2)
p-trend	----	----	0.11	0.01
p-interaction	----	----	0.15	
Recent rimming				
No	----	----	REF	REF
Yes - 1 partner	----	----	0.72 (0.45-1.2)	1.2 (0.55-2.4)
Yes - 2+ partners	----	----	1.6 (1.1-2.4)	1.7 (0.55-5.3)
p-trend			0.02	0.81
Used condom/dental dams for oral sex%				
No	----	----	REF	REF
Yes (always)	----	----	1.7 (0.96-2.9)	1.0 (0.50-1.9)
Site location				

Baltimore (MACS)	REF	----	REF	----
Chicago (MACS)	1.1 (0.82-1.4)	----	1.3 (0.87-2.0)	----
Pittsburgh (MACS)	0.93 (0.71-1.2)	----	0.62 (0.41-0.94)	----
Chicago (WIHS)	----	REF	----	REF
Bronx (WIHS)	----	0.83 (0.55-1.2)	----	0.50 (0.28-0.89)
Brooklyn (WIHS)	----	0.84 (0.54-1.3)	----	0.42 (0.23-0.75)
Ever had a tonsillectomy				
No	REF	REF	REF	REF
Yes	0.79 (0.60-1.0)	0.75 (0.56-1.0)	0.44 (0.31-0.63)	0.96 (0.58-1.6)
Unsure	0.88 (0.64-1.2)	0.87 (0.58-1.3)	1.1 (0.45-2.5)	1.3 (0.32-5.5)
Recent tooth brushing				
2 or more times per day	REF	REF	REF	REF
1 time per day	1.2 (0.86-1.6)	0.69 (0.43-1.1)	1.3 (0.90-1.9)	1.3 (0.71-2.3)
<1 time per day	0.86 (0.45-1.6)	0.87 (0.46-1.7)	1.0 (0.49-2.2)	2.8 (1.5-5.3)
No teeth/dentures	1.4 (1.1-1.9)	0.29 (0.15-0.58)	1.0 (0.36-2.8)	1.5 (0.78-2.9)
Current CD4 T cell count				
>500 cells/μL	REF	REF	REF	REF
200-499 cells/μL	0.91 (0.69-1.2)	1.0 (0.85-1.2)	1.5 (1.0-2.2)	1.3 (0.92-1.8)
≤200 cells/μL	0.65 (0.46-0.94)	1.7 (0.99-3.0)	2.3 (1.2-4.4)	4.4 (2.0-9.9)
p-trend	0.09	0.12	0.002	0.02
p-interaction	0.005		0.21	
Nadir CD4 T cell count				
500 or more cells/μL	REF	REF	REF	REF
200-499 cells/μL	1.2 (0.81-1.7)	1.1 (0.70-1.6)	1.1 (0.70-1.9)	1.1 (0.61-1.9)
200-350 cells/μL	0.90 (0.70-1.2)	1.0 (0.70-1.5)	1.9 (1.2-3.0)	1.6 (0.67-3.8)
<200 cells/μL	0.83 (0.54-1.3)	0.76 (0.56-1.0)	1.6 (0.96-2.6)	1.5 (0.84-2.7)
p-trend	0.10	0.91	0.02	0.19
HIV RNA viral load*				
Less than 50 copies/uL	REF	REF	REF	REF
50-20,000 copies/uL	1.1 (0.83-1.3)	1.0 (0.83-1.2)	1.4 (1.0-1.8)	1.4 (1.0-1.8)
20,000 or more copies/uL	0.93 (0.66-1.3)	1.4 (0.74-2.8)	2.7 (1.2-5.7)	3.5 (1.3-9.3)
p-trend	0.20	0.33	0.26	0.02
cART use				
Current	REF	REF	REF	REF
Former	0.82 (0.55-1.2)	1.7 (0.90-3.4)	1.3 (0.71-2.2)	2.2 (0.81-6.1)
Never (Naïve)	1.4 (0.97-1.9)	0.80 (0.59-1.1)	1.4 (0.75-2.5)	0.70 (0.34-1.4)

^1 negative definition of clearance

%among those performing oral sex

*less education and current hard drug use was associated with oral HPV incidence in the unadjusted model in one of the cohorts, but not the other. These variables were not significant in the combined unadjusted model or in adjusted modeling for either cohort and were thus not used in adjusted modeling.

Table 3.3 – Risk Factors related to oral HPV incidence stratified by HIV-status in the POPS

Characteristics of POPS participants	HIV-infected		HIV-uninfected	
	Unadjusted HR	Adjusted HR~	Unadjusted HR	Adjusted HR~
CD4 T cell count				
Positive CD4>500 cells/μL	REF	REF	--	--
Positive CD4 200-499 cells/μL	1.4 (1.1-1.8)	1.4 (1.1-1.7)	--	--
Positive CD4≤200 cells/μL	3.5 (1.8-6.5)	3.5 (1.7-7.3)	--	--
p-trend	0.007	0.01	--	--
Age				
Younger than 45	REF	REF	REF	REF
45-55	0.67 (0.41-1.1)	0.77 (0.52-1.1)	1.6 (0.83-3.1)	1.2 (0.62-2.4)
55 or older	0.59 (0.35-1.0)	0.59 (0.38-0.91)	1.2 (0.64-2.4)	1.1 (0.49-2.4)
p-trend	0.03	0.01^	0.61	0.84^
Gender				
Female (WIHS)	REF	REF	REF	REF
Male (MACS)	1.1 (0.73-1.5)	1.3 (0.74-2.2)	1.4 (0.85-2.3)	2.3 (0.87-6.3)
Cigarette smoking				
Never	REF	REF	REF	REF
Former	0.77 (0.45-1.3)	0.75 (0.39-1.4)	1.1 (0.64-2.1)	1.1 (0.64-1.7)
Current	1.1 (0.64-1.9)	0.86 (0.42-1.8)	1.2 (0.74-2.1)	1.0 (0.56-1.8)
Current alcohol drinking				
None	REF	REF	REF	REF
Less than 2 per day	1.1 (0.86-1.3)	0.98 (0.78-1.2)	1.0 (0.67-1.5)	0.94 (0.61-1.4)
2 or more per day	1.2 (0.59-2.5)	1.2 (0.46-3.0)	0.80 (0.39-1.6)	0.74 (0.34-1.6)
Ever had a tonsillectomy				
No	REF	REF	REF	REF
Yes	0.58 (0.41-0.81)	0.62 (0.44-0.87)	0.71 (0.41-1.3)	0.74 (0.41-1.3)
Unknown	1.1 (0.49-2.7)	0.86 (0.35-2.1)	1.5 (0.53-4.0)	0.97 (0.27-3.5)

~Incidence models adjusted for HIV-status, current CD4 T cell count, age, cigarette smoking, alcohol use, study site, history of tonsillectomy, recent tooth brushing, and lifetime and recent number of oral sex partners, and recent oral sex on a woman

^p-interaction tests were evaluated between HIV+ and HIV- individuals in adjusted model all were less than 0.05. The p-interaction for age was closest to significance (0.13).

Table 3.4 – Relationship of sexual behavior and oral HPV incidence stratified by HIV-status in the POPS

Sexual Behavior	Unadjusted HR	Adjusted HR~	Unadjusted HR	Adjusted HR~
	HIV-infected		HIV-uninfected	
Recent oral sex partners~				
0	REF	REF	REF	REF
1	0.91 (0.72-1.2)	0.84 (0.66-1.1)	0.91 (0.60-1.4)	0.84 (0.60-1.2)
2 to 5	1.4 (0.99-2.0)	1.2 (0.86-1.7)	1.6 (0.97-2.6)	1.4 (0.93-2.1)
6 or more	1.2 (0.75-2.0)	1.1 (0.62-1.9)	2.7 (1.5-4.6)	2.3 (1.1-4.8)
p-trend	0.93	0.44	<0.001	<0.001
p-interaction[&]	0.05			
Recent oral sex with on a woman[#]				
No	REF	REF	REF	REF
Yes	1.3 (0.67-2.6)	1.0 (0.54-1.8)	3.2 (1.7-6.1)	3.6 (1.8-7.0)
p-interaction[&]	0.03			
Recent number of rimming partners[#]				
None	REF	REF	REF	REF
One	0.72 (0.43-1.2)	0.72 (0.42-1.2)	1.3 (0.68-2.5)	1.7 (0.83-3.3)
Two or more	1.3 (0.81-2.1)	1.2 (0.73-1.9)	2.7 (1.5-5.0)	1.6 (0.80-3.1)
p-trend	0.96	0.92	<0.001	0.02
p-interaction[&]	0.04			
Lifetime number of oral sex partners				
0-4	REF	REF	REF	REF
5 to 99	1.7 (1.1-2.7)	2.2 (1.2-4.0)	1.5 (0.82-2.7)	1.3 (0.70-2.5)
100 or more	1.8 (1.3-2.5)	2.9 (1.6-5.2)	1.8 (1.0-3.4)	1.8 (0.80-4.3)
p-trend	<0.001	0.01	0.04	0.46
p-interaction[&]	0.76			

~The recent oral sex partners association adjusts for lifetime oral sex partners, recent oral sex on a woman and other factors in table 2, but does not adjust for recent rimming partners due to their collinearity (r=0.60). When including rimming in the model the associations between oral HPV incidence were similar but attenuated

&P-interaction tests examines the interaction between HIV-infected and HIV-uninfected individuals in adjusted analyses

#Recent oral sex on a woman and rimming (oral-anal) partners associations adjust for recent and lifetime oral sex partners along with other co-factors

Table 3.5: Risk Factors related to oral HPV incidence by gender and HIV-status

Characteristics of POPS participants	Adjusted HR (95%CI)			
	HIV+ MACS	HIV+ WIHS	HIV- MACS	HIV- WIHS
CD4 T cell count				
>500 cells/μL	REF	REF	---	---
200-499 cells/μL	1.4 (1.0-1.9)	1.3 (0.93-1.8)	---	---
≤200 cells/μL	1.9 (1.1-3.3)	4.6 (1.9-11.3)	---	---
p-trend	0.03	0.04	---	---
Age				
Younger than 45	REF	REF	REF	REF
45-55	0.71 (0.41-1.2)	0.86 (0.56-1.3)	0.73 (0.27-2.0)	1.4 (0.68-2.9)
55 or older	0.50 (0.27-0.93)	0.79 (0.49-1.3)	0.71 (0.26-2.0)	1.5 (0.64-3.8)
p-trend	0.02	0.20	0.52	0.62
Cigarette smoking				
Never	REF	REF	REF	REF
Former	0.84 (0.56-1.2)	0.74 (0.22-2.5)	0.88 (0.52-1.5)	2.0 (0.58-7.0)
Current	0.74 (0.45-1.2)	0.98 (0.30-3.2)	0.88 (0.44-1.8)	1.5 (0.43-5.2)
Ever had a tonsillectomy				
No	REF	REF	REF	REF
Yes	0.48 (0.31-0.72)	0.84 (0.47-1.5)	0.70 (0.38-1.3)	0.85 (0.21-3.4)
Unknown	1.1 (0.41-2.7)	0.79 (0.49-1.3)	1.1 (0.41-3.1)	---
Current alcohol use				
No	REF	REF	REF	REF
Less than 2 per day	0.80 (0.58-1.1)	1.1 (0.83-3.8)	0.74 (0.46-1.2)	1.1 (0.63-1.9)
Two or more per day	0.47 (0.25-0.87)	3.3 (2.0-5.6)	0.54 (0.20-1.4)	1.1 (0.43-2.7)
Recent oral sex partners				
0	REF	REF	REF	REF
1	0.67 (0.47-0.95)	1.1 (0.85-1.5)	1.0 (0.60-2.8)	0.82 (0.50-1.4)
2 to 5	1.1 (0.80-1.6)	1.7 (1.0-2.8)	1.7 (0.95-3.0)	1.4 (0.88-2.3)
6 or more	1.1 (0.63-1.8)	---	3.0 (1.3-6.7)	---
p-trend	0.64	0.44	<0.001	0.97
Recent oral sex on a woman				
No	REF	REF	REF	REF
Yes	1.3 (0.52-3.2)	0.86 (0.40-1.8)	2.8 (1.1-7.2)	4.2 (1.8-10.2)
Lifetime number of oral sex partners				
0-4	REF	REF	REF	REF
5 to 99	1.8 (0.85-3.8)	2.0 (0.96-4.0)	0.80 (0.26-2.5)	1.6 (0.75-3.3)
100 or more	2.3 (1.2-4.4)	3.0 (1.5-6.0)	1.2 (0.33-4.2)	1.0 (0.34-3.5)
p-trend	0.004	0.01	0.60	0.26
Recent rimming partners^				
0	REF	REF	REF	REF
1	0.68 (0.36-1.3)	1.0 (0.36-3.0)	1.1 (0.45-2.5)	4.6 (1.9-11.3)
2 or more	1.4 (0.82-2.3)	2.1 (0.89-5.0)	1.8 (0.84-3.7)	

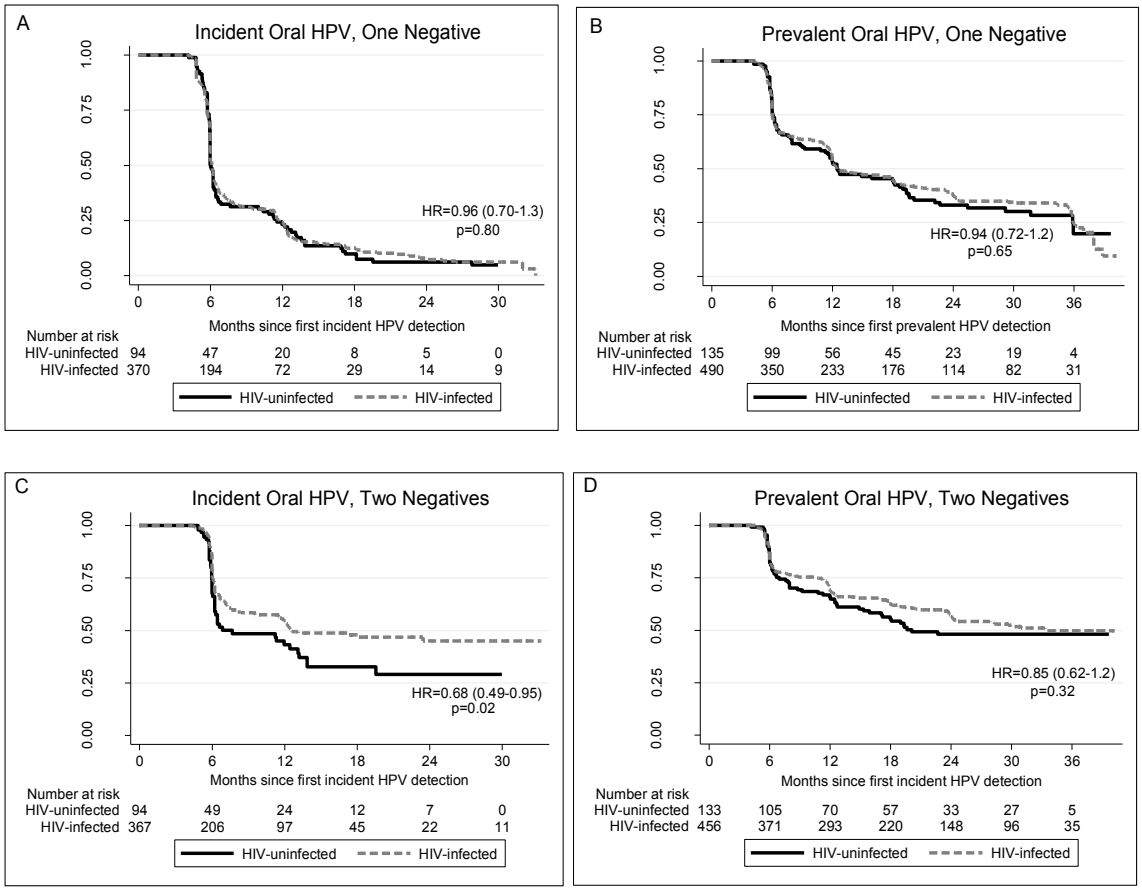
^Rimming results adjust for oral sex partners and other risk factors. However, results for the other risk factors do not adjust for number of recent rimming partners.

Table 3.6: Relationship of sexual behavior and oral HPV incidence stratified by HIV-status including adjustment for recent and lifetime number of any sexual partners

Sexual Behavior	Unadjusted HR	Adjusted HR^	Unadjusted HR	Adjusted HR^
	HIV-infected		HIV-uninfected	
Recent oral sex partners				
0	REF	REF	REF	REF
1	0.91 (0.72-1.2)	0.74 (0.56-1.0)	0.91 (0.60-1.4)	0.75 (0.49-1.1)
2 to 5	1.4 (0.99-2.0)	1.0 (0.58-1.9)	1.6 (0.97-2.6)	1.1 (0.52-2.2)
6 or more	1.2 (0.75-2.0)	0.66 (0.30-1.5)	2.7 (1.5-4.6)	2.0 (0.66-6.1)
p-trend	0.93	0.38	<0.001	<0.001
Recent sex partners (oral, anal, vaginal)				
0	REF	REF	REF	REF
1	1.2 (0.80-1.9)	1.3 (0.86-1.9)	0.82 (0.48-1.4)	1.2 (0.69-2.0)
2 to 5	1.5 (1.0-2.3)	1.3 (0.65-2.7)	1.5 (0.84-2.7)	1.5 (0.68-3.4)
6 or more	1.5 (0.97-2.5)	2.0 (0.92-4.3)	2.0 (1.1-3.8)	1.2 (0.45-3.1)
p-trend	0.72	0.21	<0.001	0.42
Recent oral sex with on a woman				
No	REF	REF	REF	REF
Yes	1.3 (0.67-2.6)	0.93 (0.48-1.8)	3.2 (1.7-6.1)	3.7 (2.0-7.1)
Lifetime number of oral sex partners				
0-4	REF	REF	REF	REF
5 to 99	1.7 (1.1-2.7)	2.1 (1.1-4.0)	1.5 (0.82-2.7)	1.6 (0.70-3.8)
100 or more	1.8 (1.3-2.5)	2.7 (1.3-5.6)	1.8 (1.0-3.4)	2.7 (0.63-11.3)
p-trend	<0.001	0.009	0.04	0.18
Lifetime number of any sex partners				
0-4	REF	REF	REF	REF
5-19	1.4 (0.76-2.5)	1.3 (0.86-2.1)	1.4 (0.55-3.3)	1.0 (0.46-2.6)
20-99	1.2 (0.80-1.8)	1.0 (0.59-1.7)	1.0 (0.39-2.8)	0.73 (0.24-2.2)
100 or more	1.4 (0.99-2.1)	1.0 (0.54-1.9)	1.5 (0.60-3.6)	0.77 (0.22-2.7)
p-trend	0.25	0.78	0.47	0.54

^Incidence models adjusted for HIV-status, current CD4 T cell count, age, cigarette smoking, alcohol use, study site, history of tonsillectomy, recent tooth brushing, and lifetime and recent number of oral sex partners, and recent oral sex on a woman, along with lifetime and recent number of any sex partners

Figure 3.2 – Clearance of incident (panels A&C) and prevalent (panels B&D) oral HPV infection comparing HIV-infected and HIV-uninfected individuals, when a single negative (panels A&B) or two consecutive negatives (panels C&D) were required for the definition of clearance in the POPS.



*HR=Hazard Ratios comparing oral HPV clearance comparing HIV-infected to HIV-uninfected individuals using Wei-Lin-Weissfeld modeling

Table 3.7 – Risk Factors related to oral HPV clearance stratified by HIV-status in the POPS

Characteristics of POPS participants	HIV-infected		HIV-uninfected	
	Unadjusted HR	Adjusted HR~	Unadjusted HR	Adjusted HR~
CD4 T cell count^				
Positive CD4>500 cells/μL	REF	REF	---	---
Positive CD4 200-499 cells/μL	0.96 (0.81-1.1)	0.98 (0.83-1.2)	---	---
Positive CD4≤200 cells/μL	1.3 (0.79-2.2)	1.1 (0.74-1.7)	---	---
p-trend	0.52	0.94	---	---
Age				
Younger than 45	REF	REF	REF	REF
45-55	0.77 (0.55-1.1)	0.87 (0.67-1.1)	0.59 (0.41-0.84)	0.60 (0.43-0.85)
55 or older	0.61 (0.43-0.88)	0.82 (0.61-1.1)	0.45 (0.31-0.63)	0.52 (0.35-0.77)
p-trend	0.003	0.18	<0.001	0.001
Gender				
Female (WIHS)	REF	REF	REF	REF
Male (MACS)	0.72 (0.57-0.92)	0.68 (0.51-0.90)	0.75 (0.56-1.0)	0.79 (0.57-1.1)
Type of infection				
Prevalent	REF	REF	REF	REF
Incident	2.6 (2.1-3.2)	2.5 (2.1-3.0)	2.2 (1.6-3.2)	2.2 (1.5-3.3)
Cigarette smoking^				
Never	REF	REF	REF	REF
Former	0.80 (0.53-1.2)	0.72 (0.48-1.1)	0.92 (0.63-1.3)	0.78 (0.54-1.1)
Current	0.86 (0.57-1.3)	0.67 (0.41-1.1)*	0.89 (0.62-1.3)	0.75 (0.49-1.1)*
Ever had a tonsillectomy				
No	REF	REF	REF	REF
Yes	0.72 (0.56-0.92)	0.83 (0.64-1.1)	0.79 (0.56-1.1)	1.0 (0.69-1.4)
Unknown	0.86 (0.63-1.2)	1.0 (0.72-1.4)	0.36 (0.15-0.82)	0.42 (0.30-0.60)

~Adjusted for current CD4 T cell count, age, gender, type of infection, cigarette smoking, history of tonsillectomy

^Effect modification by gender/cohort (p-interaction<0.10, Supp. Table 4)

*When HIV-infected and HIV-uninfected individuals were combined, oral HPV clearance was significantly lower in current smokers (p<0.05, Table 5).

#There were no significant interactions between oral HPV clearance and these risk factors by HIV-status

Table 3.8: Risk Factors for Oncogenic Oral HPV and Oral HPV16 Clearance and Incidence in the POPS

Characteristics of POPS participants	Adjusted HR (95%CI)			
	Any Oncogenic	HPV16	Any Oncogenic	HPV16
	Clearance		Incidence	
HIV-status + Current CD4				
Negative	REF	REF	REF	REF
Positive CD4>500 cells/μL	0.93 (0.70-1.2)	0.55 (0.24-1.2)	1.5 (0.98-2.2)	1.3 (0.51-3.2)
Positive CD4 200-499 cells/μL	0.89 (0.65-1.2)	0.64 (0.32-1.3)	1.9 (1.3-3.0)	1.9 (0.73-5.2)
Positive CD4<200 cells/μL	0.88 (0.52-1.5)	0.65 (0.27-1.6)	5.9 (2.7-12.8)	5.4 (1.9-15.4)
p-trend	0.56	0.39	<0.001	0.009
Age				
Younger than 45	REF	REF	REF	REF
45-55	0.96 (0.68-1.3)	0.41 (0.20-0.83)	0.85 (0.55-1.3)	0.67 (0.34-1.3)
55 or older	0.72 (0.50-1.0)	0.19 (0.08-0.45)^	0.56 (0.34-0.93)	0.50 (0.15-1.6)
p-trend	0.10	0.001	0.01	0.05
Gender				
Female (WIHS)	REF	REF	REF	REF
Male (MACS)	0.54 (0.39-0.74)	0.52 (0.26-1.1)	1.5 (0.78-2.9)	0.83 (0.17-4.0)
Type of Infection				
Prevalent	REF	REF	----	----
Incident	2.6 (2.0-3.3)	4.7 (2.5-8.7)	----	----
Cigarette smoking				
Never	REF	REF	REF	REF
Former	0.80 (0.53-1.2)	1.1 (0.43-2.6)	0.80 (0.45-1.4)	1.0 (0.28-3.5)
Current	0.55 (0.34-0.90)	0.63 (0.24-1.6)	0.87 (0.46-1.6)	2.4 (0.84-6.6)
Ever had a tonsillectomy				
No	REF	REF	REF	REF
Yes	0.72 (0.52-1.0)	0.85 (0.42-1.7)	0.70 (0.45-1.1)	1.3 (0.39-4.5)
Unsure	0.87 (0.56-1.3)	----	1.7 (0.75-4.0)	1.1 (0.18-7.0)
Current alcohol Use				
No			REF	REF
Less than 2 per day			0.91 (0.69-1.2)	0.66 (0.34-1.3)
Two or more per day			1.1 (0.57-2.2)	0.76 (0.27-2.2)
Recent oral sex partners				
0			REF	REF
1			0.76 (0.55-1.0)	1.1 (0.56-2.2)
2 to 5			1.2 (0.81-1.8)	1.6 (0.72-3.5)
6 or more			1.3 (0.78-2.3)	0.74 (0.14-4.0)
p-trend			0.56	0.19

Recent oral sex on a woman				
No			REF	REF
Yes			1.7 (1.0-3.0)	0.92 (0.19-4.5)
Lifetime number of oral sex partners				
0-4			REF	REF
5 to 99			2.3 (1.3-4.2)	3.0 (1.1-8.1)
100 or more			3.4 (1.7-6.6)	8.0 (2.1-30.3)
p-trend			<0.001	0.002

^for older age, p-interaction between HPV16 and non HPV16 types=0.02

Table 3.9: Risk Factors related to oral HPV clearance in adjusted analyses, stratified by gender and HIV-status in the POPS

Characteristics of POPS participants	Adjusted HR (95%CI)			
	HIV+ MACS	**HIV+ WIHS**	**HIV- MACS**	**HIV- WIHS**
CD4 T cell count				
>500 cells/μL	REF^	REF^	---	---
200-499 cells/μL	0.99 (0.76-1.3)	1.0 (0.84-1.2)	---	---
≤200 cells/μL	0.54 (0.37-0.78)	1.6 (1.1-2.2)	---	---
p-trend	0.001	0.04	---	---
Age*				
Younger than 45	REF	REF	REF	REF
45-55	1.0 (0.77-1.4)	0.91 (0.70-1.2)	0.54 (0.31-0.94)	0.63 (0.39-1.0)
55 or older	0.87 (0.58-1.3)	0.89 (0.61-1.3)	0.58 (0.36-0.95)	0.44 (0.22-0.87)
p-trend	0.74	0.28	0.30	0.04
Type of infection				
Prevalent	REF	REF	REF	REF
Incident	2.5 (1.9-3.3)	2.5 (2.0-3.1)	2.6 (1.7-4.1)	1.9 (0.97-3.6)
Cigarette smoker*				
Never	REF*	REF*	REF	REF
Former	0.93 (0.67-1.3)	0.43 (0.26-0.71)	0.72 (0.47-1.1)	0.71 (0.29-1.7)
Current	1.0 (0.74-1.4)	0.38 (0.26-0.71)	0.69 (0.41-1.2)	0.78 (0.32-1.9)
Ever had a tonsillectomy				
No	REF	REF	REF	REF
Yes	0.84 (0.59-1.2)	0.74 (0.51-1.1)	0.80 (0.51-1.3)	1.7 (1.2-2.4)
Unknown	1.1 (0.74-1.5)	0.75 (0.36-1.6)	0.32 (0.22-0.48)	--

^p-interaction for CD4 T cell count between MACS and WIHS is 0.07

*p-interaction for current smoking between MACS and WIHS is 0.01

Table 3.10: Risk Factors related to oral HPV prevalence, incidence, and clearance among all 1,230 POPS participants

Characteristics of POPS participants	Adjusted OR	Adjusted HR (95%CI)		
	Baseline Prevalence~	Incidence~	Clearance (1 negative)*	Clearance (2 negative)%
HIV-status + Current CD4 (cells/ μL)				
Negative	REF	REF	REF	REF
Positive CD4>500	1.9 (1.4-2.6)	1.7 (1.3-2.3)	0.90 (0.75-1.1)	0.76 (0.58-0.99)
Positive CD4 200-499	2.6 (1.9-3.6)	2.4 (1.7-3.2)	0.87 (0.72-1.1)	0.78 (0.60-1.0)
Positive CD4<200	4.2 (2.8-6.3)	6.0 (2.7-13.3)	1.0 (0.64-1.6)	0.71 (0.50-0.99)
p-trend	<0.001	<0.001	0.94	0.42
Age				
Younger than 45	REF	REF	REF	REF
45-55	0.92 (0.67-1.3)	0.89 (0.63-1.2)	0.80 (0.64-1.0)	0.88 (0.70-1.1)
55 or older	1.2 (0.83-1.7)	0.68 (0.47-0.99)	0.73 (0.58-0.93)	0.72 (0.54-0.95)
p-trend	0.26	0.05	0.04	0.004
Gender				
Female (WIHS)	REF	REF	REF	REF
Male (MACS)	0.81 (0.49-1.3)	1.5 (0.92-2.3)	0.69 (0.54-0.89)	0.84 (0.67-1.1)
Type of Infection				
Prevalent	---	---	REF	REF
Incident	---	---	2.4 (2.0-2.8)	1.6 (1.3-2.0)
Cigarette smoking				
Never	REF	REF	REF	REF
Former	1.6 (1.1-2.2)	0.83 (0.49-1.4)	0.73 (0.53-1.0)	0.87 (0.67-1.1)
Current	2.8 (2.0-3.7)	0.88 (0.47-1.6)	0.67 (0.45-0.99)	0.91 (0.69-1.2)
Ever had a tonsillectomy				
No	REF	REF	REF	REF
Yes	1.0 (0.74-1.3)	0.62 (0.46-0.83)	0.86 (0.69-1.1)	0.91 (0.69-1.2)
Unknown	2.0 (0.87-4.7)	0.92 (0.40-2.1)	0.86 (0.65-1.2)	1.5 (1.1-2.0)
Current alcohol use				
None	REF	REF		
Less than 2 drinks/day	0.73 (0.56-0.94)	0.95 (0.78-1.2)		
2 or more drinks/day	0.59 (0.36-0.98)	1.0 (0.50-2.2)		
p-trend	0.004	0.94		
Recent oral sex partners				
0	REF	REF		
1	0.88 (0.62-1.2)	0.79 (0.63-1.0)		
2 to 5	1.2 (0.80-1.7)	1.2 (0.91-1.7)		
6 or more	1.8 (1.0-3.0)	1.3 (0.84-2.0)		

p-trend	0.002	0.33		
Recent oral sex on a woman				
No	REF	REF		
Yes	2.3 (1.4-3.8)	1.9 (1.2-2.9)		
Lifetime number of oral sex partners				
0-4	REF	REF		
5 to 99	1.3 (0.95-1.7)	1.9 (1.1-3.2)		
100 or more	1.9 (1.2-2.7)	2.6 (1.6-4.3)		
p-trend	0.005	<0.001		

*Clearance models adjusted for HIV-status, current CD4 T cell count, age, type of infection, cigarette smoking, and history of tonsillectomy

~Prevalence and incidence models were adjusted for HIV-status, current CD4 T cell count, age, cigarette smoking, study site, history of tonsillectomy, recent tooth brushing, current alcohol use, lifetime and recent number of oral sex partners, and recent oral sex on a woman

%Two consecutive negatives were necessary for an infection to be considered cleared utilizing this definition

Chapter 4: Incidence and risk factors of HPV-related and HPV-unrelated Head and Neck Squamous Cell Carcinoma in HIV-infected individuals

Incidence and risk factors of HPV-related and HPV-unrelated Head and Neck Squamous Cell Carcinoma in HIV-infected individuals

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Abstract:

Background: Head and neck squamous cell carcinoma (HNSCC) is a heterogeneous malignancy caused by HPV infection, alcohol, and tobacco use, and is elevated among HIV-infected individuals. It is unclear if and when in the carcinogenesis process HIV-related immunosuppression might play a role in HNSCC development.

Methods: Data from 17 prospective cohort studies were utilized from the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD). A standardized process of chart review and cancer registry matching was used to validate incident HNSCCs occurring in the HIV-infected participants between 1996-2009. HNSCCs were analyzed by stratifying the cancers into HPV-related and HPV-unrelated subsites, and rates were compared to a Surveillance Epidemiology and End Results general population estimate. Risk factors for incident HPV-related and HPV-unrelated HNSCC were explored using mixed effects Poisson regression in a full prospective analysis, and the effect of CD4 prior to cancer diagnosis was examined retrospectively in a nested case control analysis.

Results: 66 HPV-related and 182 HPV-unrelated incident HNSCCs were detected among 82,375 HIV-infected participants. The standardized incidence of both HPV-related (SIR=3.0, 95%CI=2.3-3.8) and HPV-unrelated (SIR=3.6, 95%CI=3.1-4.1) HNSCC was significantly higher among these HIV-infected individuals compared with the US general population. Between 1996 to 2009, HPV-related HNSCC incidence increased non-significantly, while incidence of HPV-unrelated HNSCC decreased non-significantly. Lower CD4 T cell count measured up to seven years prior to cancer diagnosis was significantly associated with increased HPV-related and HPV-unrelated HNSCC.

Conclusions: The incidence of HPV-related and HPV-unrelated HNSCC are elevated in HIV-infected individuals. Immunosuppression may play a role in the development of both HPV-related and HPV-unrelated HNSCC.

Introduction:

Human papillomavirus (HPV), tobacco, and alcohol use are three major causes of head and neck squamous cell carcinoma (HNSCC).[1,2] In the general population of North America, the incidence of many types of HNSCCs, such as oral cavity cancer, have been decreasing over the past several decades, due to decreases in tobacco use.[3,4] In contrast, incidence of HNSCCs related primarily to oral HPV infection, such as oropharyngeal cancer, have been increasing over the same time period.[3-5]

Less is known about HNSCC in the HIV-infected population of North America, as few studies have been large enough to explore this heterogeneous malignancy. Large cohort and HIV/AIDS-Cancer registry match studies have suggested that HIV-infected individuals have a 1.5 to 4 fold higher risk for HNSCC compared with the general population.[6-8] However, many of these studies have categorized HPV-related and HPV-unrelated HNSCCs together in one "oral cavity and pharynx cancer" category, so it is unclear whether this increased HNSCC risk may be explained by higher levels of tobacco and alcohol use, higher exposure to HPV, faster progression of HPV-related carcinogenesis, or a combination of these factors.[9,10]

The incidence of several cancers caused by infectious agents, such as Kaposi's sarcoma, has decreased dramatically in HIV-infected individuals after the introduction of effective antiretroviral therapy (ART, also known as HAART or cART) around 1996. In contrast, there is evidence that the incidence of HPV-related cancers such as anal cancer,[11,12] and perhaps oropharyngeal cancer may be increasing in HIV-infected individuals over the past two decades.

The role of immunosuppression in the development of HNSCC is unclear as some preliminary studies have suggested an association with increased HNSCC risk,[13-15] but another did not.[16] Using data from the NA-ACCORD (North American AIDS Cohort Collaboration on Research and Design),[17] a collaboration of longitudinal studies of HIV-infected individuals, we explored the risk and trends of HPV-related and HPV-unrelated HNSCC, and assessed the degree to which immunosuppression plays a role in increasing HNSCC risk.

Methods:

Study Population and Design:

The NA-ACCORD is a large collaboration of longitudinal cohort studies involving HIV-infected individuals in North America and is a part of the International Epidemiologic Databases to Evaluate AIDS (IeDEA) initiative.[17] HNSCCs were identified and validated from three interval and fourteen clinically based cohort studies that obtain data from patient medical records. We included HIV-infected individuals from these 17 cohort studies who contributed data to the NA-ACCORD between 1996 and 2009**.** Individual cohorts in the NA-ACCORD each developed their own standardized methods of data collection. Briefly, each cohort submits demographic, treatment, clinical, and laboratory data to the NA-ACCORD data management center. The data management center harmonizes the individual cohort datasets and implements a series of rigorous quality control procedures.

Participant's follow-up time for this study began at the maximum of: January 1, 1996, the entry into the cohort or the earliest date of cancer validation. Participants were followed either until the minimum of: the date of their HNSCC diagnosis, loss to follow-up, death, a cohort's specific administrative censoring date or the last date of cancer validation

(December 31, 2009 for most cohorts). Data from the Surveillance Epidemiology and End Results (SEER) program on anatomical site-specific HNSCC was utilized as a general population comparison.[18]

Data in this analysis was restricted to the ART era (1996 or later), but included both individuals who used and did not use ART. ART use was defined as a regimen involving at least three antiretrovirals, including a protease inhibitor, an entry inhibitor or an integrase inhibitor, or three nucleoside reverse-transcriptase inhibitors, including abacavir or tenofovir based on US Department of Health & Human Services/Kaiser Panel.[19] Institutional review boards at each of the participating cohort site locations have reviewed and approved the human subject activities of the NA-ACCORD.

Cancer validation and categorization:

HNSCC cases were validated before this analysis through chart review with clinical confirmation from medical records/pathology reports or through cancer registry-linkage. A standardized abstraction survey was utilized to review each HNSCC case at 16 of the 17 contributing cohort studies. The survey included histologic confirmation of each cancer along with the source of cancer confirmation and the date of diagnosis. One cohort (hereby called cohort A) used its own internal validation system which adheres to the North American Association of Central Cancer Registries quality standards. They also completed an additional validation process on a random subset of cases with strong sensitivity (87%) and positive-predictive value (96%).

All HNSCCs were included in the analysis.[20] These included all cancers classified as C00.0-C14.8, C30.0-C31.9, and C32.0-32.9 using the International Classification of Disease for Oncology version-3 (ICD-0-3) topography codes. As tumor HPV status was not

available in this study, all oropharyngeal cases were considered "HPV-related" in this analysis, as this assumption is supported by data indicating oncogenic HPV is detectable in approximately 50-80% of US individuals with oropharyngeal cancer.[3,5] HPV-related HNSCCs were defined using the following ICD-0-3 topographic codes: base of the tongue (C01.9), lingual tonsil (C02.4), palatine tonsil (C09.0-09.9), oropharynx (C10.2-10.9), pharynx NOS (C14.0), and Waldyer's ring (C14.2).[3] All other HNSCCs were classified as HPV-unrelated including: lip (C00.0-00.9), oral cavity (C02.0-02.3 and C02.5-06.9), hypopharynx (C12.9-13.9), nasopharynx (C11.0-11.9), salivary gland (C07.9-08.9), anterior epiglottis (C10.1), other overlapping sites (C14.8), nasal cavity and middle ear (C30.0-30.1), accessory sinuses (C31.0-31.9), and larynx (C32.0-32.9) cancers. For these analyses, cancers were restricted to squamous cell carcinomas, histologic ICD-O-3 codes (8050-76, 8078, 8083, 8084, 8094) and those labeled carcinoma, NOS (8010).

Statistical Analysis:

The characteristics of participants who developed an incident HPV-related or HPV-unrelated HNSCC were compared to each other and with all other NA-ACCORD participants who did not develop an HNSCC using χ^2 tests for categorical data and Wilcoxon-Mann-Whitney test for medians for continuous data. Incidence rates of HPV-related and HPV-unrelated HNSCC were calculated among HIV-infected individuals overall (from 1996 to 2009) and were stratified by calendar period. In order to compare subgroups with each other, such as the different calendar periods, we performed direct age standardization of these rates using the 2000 US census standard population and restricted to ages 20 or older in order to better match the NA-ACCORD population.[18] To compare the rate of HNSCC in the NA-ACCORD with the US general population, standardized

incidence ratios (SIRs) were also calculated using indirect standardization using rates from Surveillance Epidemiology and End Results (SEER) from 1996-2009.[18]

Mixed effects Poisson regression was utilized to model the incidence rates and examine risk factors for HNSCC incidence. A random intercept was used to account for differences in HNSCC incidence by cohort. All multivariate Poisson models included age, gender, smoking status and baseline CD4 T cell count.

We also explored the effect of CD4 T cell count prior to cancer diagnosis utilizing a nested case control study similar to previous studies.[21,22] We matched each HNSCC case to up to ten controls on age (+5 years), cohort, sex, and enrollment year in the study. As incident density sampling was utilized, the controls had the same amount of study follow-up time as the cases. Reference dates were defined as the date of cancer diagnosis for HNSCC cases, or for controls as the date after the same length of follow-up time in the NA-ACCORD as the matched HNSCC case. To evaluate the impact of CD4 prior to the reference date, we conducted an additional analysis categorizing CD4 measures into time periods categories prior to the reference date (less than 2 years, 2-4 years, 5-7 years, or 8-10 years). The results were then evaluated using conditional logistic regression. For this analysis, if more than one CD4 cell count measurement was available for an individual case or control in a specific time period, then the CD4 measurement closest to the reference date was used.

Data on participant's cigarette smoking status was non-uniformly collected by all participating cohorts, and in one cohort (cohort A, the largest cohort in the NA-ACCORD), no smoking information was available on any participants. Overall, smoking status was available on 40% of the participants in our study, as 47% of our total NA-ACCORD sample was from cohort A, and 13% of the sample was missing smoking data and was from cohorts

other than cohort A. To account for this missingness in the ever smoking variable in these 13% of individuals, stochastic multiple imputation via logistic regression was performed.[23] The binomial proportion of the time-fixed smoking variable was calculated through MIANALYZE from SAS and utilized other available covariates that may be associated with smoking status: study site, age, sex, race, HIV transmission risk, death during study, follow-up time, CD4 and HIV RNA at entry, and ever ART use. Five random draws from this binomial distribution were averaged to determine the ever smoking estimate for each individual that was missing smoking information and to acquire the proper standard errors. We did not impute the smoking status for participants in cohort A as the imputation largely relied on having at least some participants with smoking data from the same study site.

Results:

Participant Characteristics:

This study includes 82,375 HIV-infected participants from 17 cohort studies in the NA-ACCORD. These participants contributed 463,784 person-years between 1996 and 2009, with a median follow-up time per participant of 4.9 (IQR=2.0-8.9) years. Only a subset of participants were on ART at entry into the study (20%), however most of the study participants had been exposed to ART by the end of the study (78%). There were 248 incident HNSCCs ascertained throughout this study. These HNSCC cases were more likely to be older, male, African American, ever smokers, and have a lower CD4 T cell count compared with other individuals in the NA-ACCORD (Table 4.1, all p-values<0.01). Characteristics of individuals who developed HPV-related HNSCC were similar to those who developed HPV-unrelated HNSCCs, except individuals with HPV-related HNSCC were modestly younger. (Table 4.1, p=0.04).

Incidence of HPV-related and HPV-unrelated HNSCC

In the HIV-infected participants of NA-ACCORD, the age standardized incidence rate for overall HNSCC was 50.6 (95%CI=41.0-60.1) per 100,000 person-years. The anatomical subsites of the incident HNSCC cases in the HIV-infected individuals from the NA-ACCORD are described in Table 4.2. Among the 248 incident HNSCCs, there were 182 HPV-unrelated HNSCCs and 66 HPV-related HNSCCs. The majority of HPV-unrelated HNSCCs were larynx and oral cavity cancers, while the most common anatomical sites for HPV-related HNSCCs were the lateral wall of the oropharynx and the base of the tongue (Table 4.2). The age-standardized incidence of HPV-unrelated HNSCC (40.0, 95%CI=31.0-49.0 per 100,000 person-years) was significantly higher than HPV-related HNSCC (10.7, 95%CI=7.5-13.9 per 100,000 person-years).

Compared with the general US population from SEER, HIV-infected individuals in the NA-ACCORD had a three-fold higher standardized incidence of HNSCC (SIR=3.4, 95%CI=3.0-3.8, Table 4.2). The three-fold increased incidence of cancer was consistent across all head and neck anatomical sites (Table 4.2), including sites related to HPV (SIR=3.0, 95%CI=2.3-3.8) and those not related to HPV (SIR=3.6, 95%CI=3.1-4.1).

Incidence of HNSCC over time:

The incidence of HNSCC did not substantially change over the course of this study, but there were non-significant trends which appeared to mirror the trends in the US general population (Figure 4.1). The age-standardized incidence of HPV-related HNSCC was close to twice as high in 2001-2005 (IR=12.4 per 100,000, 95%CI=7.4-17.6) compared with 1996-2000 (6.8 per 100,000, 95%CI=1.3-12.3), although this difference was not statistically significant and did not continue to increase in more recent years (Figure 4.1). The age-

standardized incidence of HPV-unrelated HNSCC remained constant between 1996 and 2005, and then non-significantly declined in more recent years (48.6 vs. 29.3 per 100,000, Figure 4.1).

Impact of risk factors on HNSCC incidence:

Risk factors were similar for incidence of HPV-related and HPV-unrelated HNSCC. Older age appeared to have the strongest impact on HNSCC incidence, as older participants had a significantly higher incidence of HPV-related HNSCC, as well as HPV-unrelated HNSCC, after adjustment for other risk factors(both $p<0.001$, Table 4.3). In addition, ever smokers and those with a lower baseline CD4 T cell count both had an non-significantly elevated incidence of HPV-related and HPV-unrelated HNSCC after adjustment (Table 4.3).

The incidence of both HPV-related and HPV-unrelated HNSCC were similar among men who have sex with men, other males, and females. In addition, HPV-related and HPV-unrelated HNSCC incidence was similar by race, baseline HIV RNA viral load, and baseline CD8 T cell count. Those not on ART at baseline had a similar incidence of HPV-related HNSCC, but a lower incidence of HPV-unrelated HNSCC compared to those on ART at baseline.

In a sensitivity analysis, we considered the effect of risk factors on HNSCC incidence by cohort and observed modest differences between the cohort A and the other cohorts in the NA-ACCORD (Table 4.4). We found that, qualitatively, reduced baseline CD4 was more strongly associated with HPV-related HNSCCs than HPV-unrelated HNSCCs in cohort A, while in the other cohorts, reduced CD4 was more strongly associated to HPV-unrelated HNSCCs. However, these interactions did not reach statistical significance (p-interactions$\geq$0.20, Table 4.4). To determine if this potential difference by cohort was due to

the lack of smoking data in the cohort A, we ran another sensitivity analysis that excluded the participants in cohort A. We found that adjusting for ever smoking status did not strongly impact the point estimates between risk factors and HPV-related or HPV-unrelated HNSCC in the non-cohort A studies (Table 4.5). In addition, we found that the baseline CD4 T cell count in ever and never smokers was similar (p=0.90).

Impact of CD4 cell count on HNSCC incidence:

After finding that reduced baseline CD4 T cell count may be associated with increased HNSCC incidence, we further explored the effect of reduced CD4 by conducting a nested case control analysis. This nested case-control analysis considered CD4 measures in cases and controls prior to a reference date, which was defined as the date of cancer diagnosis for cases or the date after the same length of follow-up in the NA-ACCORD as the matched HNSCC case for controls. When measured up to seven years prior to the reference date, reduced CD4 T cell count measured was associated with HPV-related and HPV-unrelated HNSCC (Figure 4.2, Table 4.6). CD4 cell count measured within two years prior the reference date was associated with both HPV-related (CD4<200: aOR=2.3, 95%CI=1.1-4.8) and HPV-unrelated HNSCC (CD4<200: aOR=3.3, 95%CI=2.0-5.6). Similar associations were seen when CD4 was measured up to seven years prior to the reference date for both HPV-related and HPV-unrelated HNSCC (Table 4.6), although there was a suggestion that the association may have strengthened further from the reference date for HPV-related HNSCC (5-7 years, CD4<200: aOR=5.1, 95%CI=1.5-17.3).

Discussion:

In this large prospective study, we determined that the incidence of HPV-related and HPV-unrelated HNSCC were both threefold higher in HIV-infected individuals than the US

general population. This study also supports a modest role for immunosuppression prior to cancer diagnosis, which suggests when paired with other studies,[7,24] that immunosuppression from HIV may have some impact during the oral carcinogenesis process.

The three fold higher incidence of HNSCC in this HIV-infected population compared with the general population is consistent with previous registry based studies which have suggested that the incidence of HNSCC is between 1.5 and 4 fold higher in HIV-infected individuals.[6,7] In addition, the incidence of HPV-related HNSCC increased while the incidence of HPV-unrelated HNSCC decreased among these HIV-infected individuals between 1996 and 2009, which mirrors what has been observed in the general HIV-uninfected population.[3,5] However, the calendar trends in this study were modest and did not reach statistical significance. This may, in part, be explained by the imprecise groupings of cancers in our study by tumor subsite, since HPV status was unavailable; as some cases in the HPV-related HNSCC group were likely HPV-negative this misclassification may have attenuated actual trends.

It has been speculated that the higher incidence of HNSCC in HIV-infected individuals might be due to multiple different factors including: their increased use of tobacco or alcohol, their higher number sexual partners, and/or from HIV-related immunosuppression.[7,25] However, the effect of immunosuppression on HNSCC incidence has not been extensively explored previously. Most previous studies that include HNSCCs in HIV-infected individuals have been limited to exploring the role of CD4 T cell count at one time point, and have not explored the effect immunosuppression trends over time.[14,15] Our study supports the conclusion that immunosuppression may have a modest role in the

development of HNSCC,[13-15] and suggests this may be true for both HPV-related and HPV-unrelated HNSCC.

Our finding that HPV-related HNSCC is associated with immunosuppression measured up to seven years prior to cancer diagnosis, is consistent with other studies suggestion immunosuppression is associated with higher incidence of HNSCC[13-15] and with increased risk of acquisition of oral HPV infection (Beachler unpublished). It has been previously speculated that immunosuppression may have its strongest impact earlier in the HPV-related carcinogenesis process[26] however; initial finds exploring the longitudinal effect of CD4 on other HPV-associated cancers have been mixed. One recent study suggested that immunosuppression may have its largest effect 6-7 prior to *anal* cancer diagnosis,[22,26] while another study suggested a stronger effect close to *cervical* cancer diagnosis.[21] The results of this NA-ACCORD study found that lower CD4 measured further from HPV-related HNSCC diagnosis (5-7 years vs. 0-2 years) may be more strongly associated with HPV-related HNSCC; however the difference between these CD4 measures was not significant.

The finding that immunosuppression was also similarly associated with HPV-unrelated HNSCC in this study suggests that reduced immune surveillance for malignant cells could have a role in HNSCC development.[27,28] There have been several recent studies that have suggested immunosuppression closer to cancer diagnosis may impact infection and non-infection related non-AIDS defining malignances.[9,13,29,30] For example, a study from Kaiser Permante[13] suggested that reduced recent CD4 was associated with a higher risk of both infection related and non-infection related cancers, except prostate cancer. Alternatively, it is also conceivable that undiagnosed HNSCC may be playing a role in reducing a participant's immune status. A recent European study found a higher risk of

cancer diagnosis within six months of a detectable decline in CD4 count, suggesting that the decline in CD4 may be a consequence of the cancer rather than a causal agent in the malignancy's development.[31]

While we note a modest association between immunosuppression and HPV-related HNSCC risk, the three fold higher SIR for HPV-related HNSCC (comparing HIV-infected individuals with the general population) is considerably lower than the SIRs for other HPV-associated cancers including cervical, anal, and vulvar cancer (SIRs>5).[6,14,32] It is possible that the SIR of HPV-related HNSCC may be lower due to the demographics and sexual orientation of the North American HIV-infected population.[6,7] Specifically, the incidence of HNSCC may be modest in North American HIV-infected individuals as ~75% are either heterosexual women or MSM[33] and there is evidence that performing oral sex on a man may be *less* likely to transmit oral HPV infection than performing it on a woman.[7,34] However in this study heterosexual men had a similar incidence of HPV-related HNSCC compared to MSM and women, although we were unable to adjust for important risk factors such as number of lifetime oral sex partners.

There are several strengths to this study. The study used prospective data from a large collaboration of HIV-studies that have been suggested to be representative of the HIV-infected population in clinical care in North America.[17] In addition, this study included a rigorous standardized validation procedure to confirm malignancies, included approximately 15 years of data, and is one of the first studies to explore HPV-related and HPV-unrelated HNSCC incidence among HIV-infected individuals. However, we should note that we lacked detailed information on other co-factors such as sexual behavior, alcohol use, and detailed tobacco use (such as current tobacco use and cumulative tobacco use) and thus the

risk factor's associations with HNSCC are still prone to residual confounding. In addition, there was outcome misclassification as all oropharyngeal cancers were classed HPV-related in this study. While a recent case series suggests that HPV is detected in most oropharyngeal cancers in HIV-infected individuals,[35] there were still likely HPV-negative oropharyngeal cancers that were classified as HPV-related HNSCCs in this study. This may impact both the HPV-related SIR and the association of risk factors, although results were relatively similar between the HPV-related and HPV-unrelated HNSCCs in this study.

This study demonstrates that North American HIV-infected individuals have an elevated risk of both HPV-related and HPV-unrelated HNSCC. However, these SIRs are relatively modest compared to other virally induced carcinomas, suggesting that the North American HIV-infected population as a whole may not be strong candidates for more aggressive HNSCC screening modalities. While there has not been a strong decline in HNSCC incidence in the antiretroviral therapy era, these results and others suggest that immunosuppression may play a role during the HNSCC carcinogenesis process, which suggests that ART may provide some benefit in preventing this disease.

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Table 4.1: Characteristics of the participants in NA-ACCORD compared with those with HPV-related and HPV-unrelated Head and Neck Squamous Cell Carcinomas (HNSCCs)

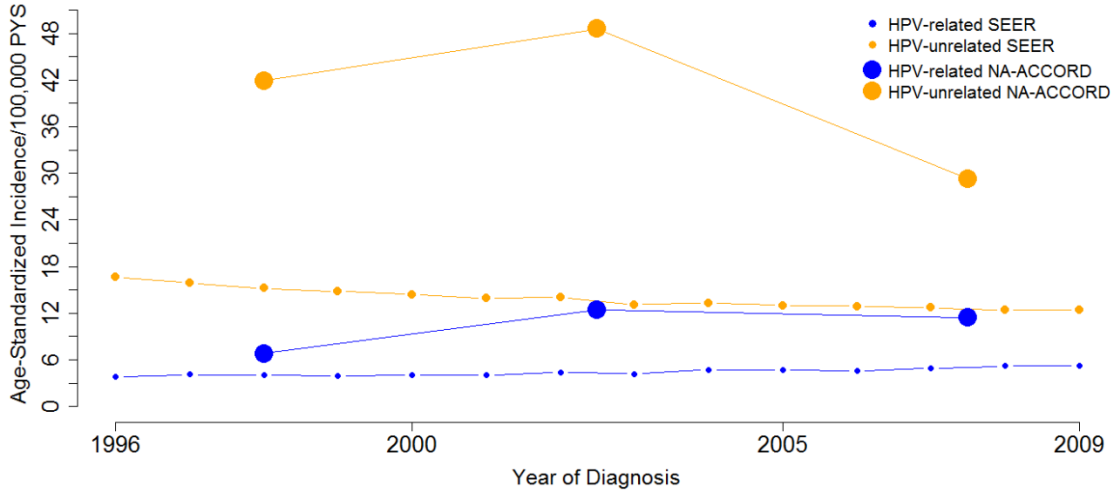
	HIV-positive individuals in NA-ACCORD		
	Entire Cohort (n=82,375)	HPV-related HNSCC (n=66)	HPV-unrelated HNSCC (n=182)
Median Age at Baseline (IQR)	42.8 (36-49.7)	48.5 (43.4-52.3)	49.0 (44.4-55.3)
Gender/Sexual Orientation			
Female	11,308 (14%)	5 (7.6%)	14 (7.6%)
Male (heterosexual and MSM)	71,308 (86%)	61 (92.4%)	168 (92.3%)
MSM only*	20,960 (50%)*	8 (47%)	15 (40%)
Race			
Black (non-Hispanic)	31,411 (38%)	35 (53%)	86 (47%)
White	36,588 (47%)	24 (36%)	91 (50%)
Other	13,897 (17%)	7 (10%)	5 (2.8%)
Median Year of Entry (IQR)	2000 (1997-2004)	1999 (1997-2000)	1998 (1997-1998)
Median Years of Follow-up (IQR)	4.9 (2.0-8.9)	4.7 (3.7-6.0)	4.5 (3.6-5.1)
Median CD4 T Cell Count at Baseline (IQR)	293 (127-487)	248 (189-327)	240 (205-322)
Current ART use at baseline	15,990 (20%)	12 (19%)	44 (24%)
Ever ART during Study	64,072 (78%)	54 (82%)	137 (75%)
Median HIV Viral load at Baseline (IQR)	15258 (508-91000)	9479 (400-63608)	15,100 (971-78032)
$\leq$500 copies/mL	14,388 (18%)	15 (23%)	17 (16%)
>500 copies/mL	64,587 (81%)	51 (77%)	92 (84%)
Smoking*			
Ever	31,857 (76%)	21 (84%)	44 (92%)
Never	10,016 (24%)	4 (16%)	4 (8.3%)
Stage of Cancer			
T1-T2	n/a	38 (58%)	118 (64%)
T3-T4	n/a	19 (29%)	32 (18%)
Unknown	n/a	9 (14%)	32 (18%)

*missing from cohort A (47 % of the study population)

Table 4.2: Head and Neck Squamous Cell Carcinomas in NA-ACCORD compared to the US General Population between 1996-2009

Anatomical Site	Observed Total	Standardized Incidence Ratio (SIR)
Total Head Neck Squamous Cell Carcinomas (HNSCCs)	**248**	**3.4 (3.0-3.8)**
HPV-related – Oropharynx	**66**	**3.0 (2.3-3.8)**
Oropharynx - Lateral Wall/Tonsil/Posterior Wall/Unspecified	43	3.4 (2.4-4.5)
Base of Tongue	20	2.3 (1.4-3.6)
Pharynx, NOS	4	4.3 (1.2-11.0)
HPV-unrelated	**182**	**3.6 (3.1-4.1)**
Larynx/Hypopharynx/Supraglottis	95	4.2 (3.4-5.2)
Oral Cavity	56	3.0 (2.3-3.9)
Lip	10	2.9 (1.4-5.3)
Paranasal sinus/Nasal cavity/Middle ear	9	5.3 (2.4-10.1)
Nasopharynx	8	3.3 (1.0-4.8)
Salivary Gland	4	3.7 (1.0-9.7)

Figure 4.1: Age Standardized Incidence Rates by Year of Diagnosis by calendar time between 1996-2009



Age Standardized Incidence Rates per 100,000 person-years among the HIV-positive participants in the NA-ACCORD		
Calendar Period	HPV-unrelated	HPV-related
1996-2000	41.9 (27.8-56.0)	6.8 (1.3-12.3)
2001-2005	48.6 (31.1-66.1)	12.4 (7.1-17.6)
2006-2009	29.3 (19.1-39.5)	11.4 (5.6-17.1)
Overall	40.0 (31.0-49.0)	10.7 (7.5-13.9)

Table 4.3: Unadjusted and adjusted incidence rate ratios for risk factors for HPV-related and HPV-unrelated Head and Neck Squamous Cell Carcinoma (HNSCC)

Variable	HPV-related HNSCC		HPV-unrelated HNSCC	
	IRR	aIRR^	IRR	aIRR^
Age				
<40	REF	REF	REF	REF
40-50	3.4 (1.6-7.1)	3.3 (1.5-7.6)	4.9 (2.8-8.2)	4.7 (2.6-8.5)
50+	4.2 (1.9-9.2)	4.9 (2.0-11.9)	8.5 (4.9-14.5)	7.3 (3.9-13.7)
Gender				
Female	REF	REF	REF	REF
Male	1.6 (0.55-4.3)	1.1 (0.40-3.0)	1.7 (0.81-3.7)	1.1 (0.50-2.2)
MSM^	1.0 (0.32-3.1)	---	0.88 (0.35-2.2)	---
Other Men^	1.1 (0.29-4.1)	---	1.0 (0.41-2.6)	---
Race				
Black	REF	---	REF	---
White	0.69 (0.41-1.2)	---	1.3 (0.78-1.4)	---
Other	0.63 (0.24-1.7)	---	0.23 (0.09-0.60)	---
Smoking status				
Never	REF	REF	REF	REF
Ever	2.5 (0.57-10.8)	2.5 (0.57-11)	3.0 (1.1-8.4)	2.7 (0.96-7.6)
ART use at baseline				
Yes	REF	---	REF	---
No	1.0 (0.53-1.8)	---	0.68 (0.48-0.96)	---
Baseline CD4				
≥500 cells/uL	REF	REF	REF	REF
200-500 cells/uL	2.0 (0.86-4.7)	1.9 (0.82-4.4)	1.7 (0.97-2.8)	1.6 (0.92-2.7)
<200 cells/uL	1.8 (0.74-4.5)	1.6 (0.82-4.4)	1.9 (1.1-3.3)	1.7 (0.98-2.3)
Baseline HIV viral load				
<500 copies/mL	REF	---	REF	---
500-10000 copies/mL	0.49 (0.22-1.1)	---	0.74 (0.41-1.3)	---
>10000 copies/mL	0.49 (0.23-1.0)	---	0.94 (0.55-1.6)	---
Baseline CD8				
Highest (Quartile 1)	REF	---	REF	---
Quartile 2	2.0 (0.37-11.0)	---	0.42 (0.13-1.4)	---
Quartile 3	2.1 (0.38-11.7)	---	0.74 (0.27-2.0)	---
Quartile 4	1.1 (0.15-7.5)	---	0.74 (0.28-2.0)	---

^Adjusted for age, gender, smoking status, CD4 at baseline, and cohort

Table 4.4 – The effect of risk factors on HPV-related and HPV-unrelated HNSCC stratified by whether a participant is a part of the Cohort A

Covariate	IRR~			
	HPV-related HNSCC		HPV-unrelated HNSCC	
	All Others Cohorts	Cohort A	All Other Cohorts	Cohort A
Age				
<40 years old	REF	REF	REF	REF
40-50 years old	2.2 (0.81-6.1)	5.2 (1.6-17.1)	7.7 (3.1-19.0)	3.2 (1.7-6.3)
50 years old or older	3.1 (0.91-10.6)	5.1 (1.5-17.5)	14.0 (5.2-37.3)	5.7 (3.0-10.8)
p-interaction*	0.29		0.35	
Sex				
Female	REF	REF	REF	REF
Male	1.0 (0.36-1.8)	---^	0.84 (0.37-1.9)	---^
Baseline CD4				
≥500 cells/uL	REF	REF	REF	REF
200-500 cells/uL	1.8 (0.58-5.8)	2.1 (0.60-7.6)	4.6 (1.6-13.3)	0.90 (0.48-1.7)
<200 cells/uL	0.91 (0.23-3.6)	2.7 (0.76-9.6)	2.7 (0.85-8.8)	1.5 (0.82-2.8)
p-interaction*	0.21		0.53	

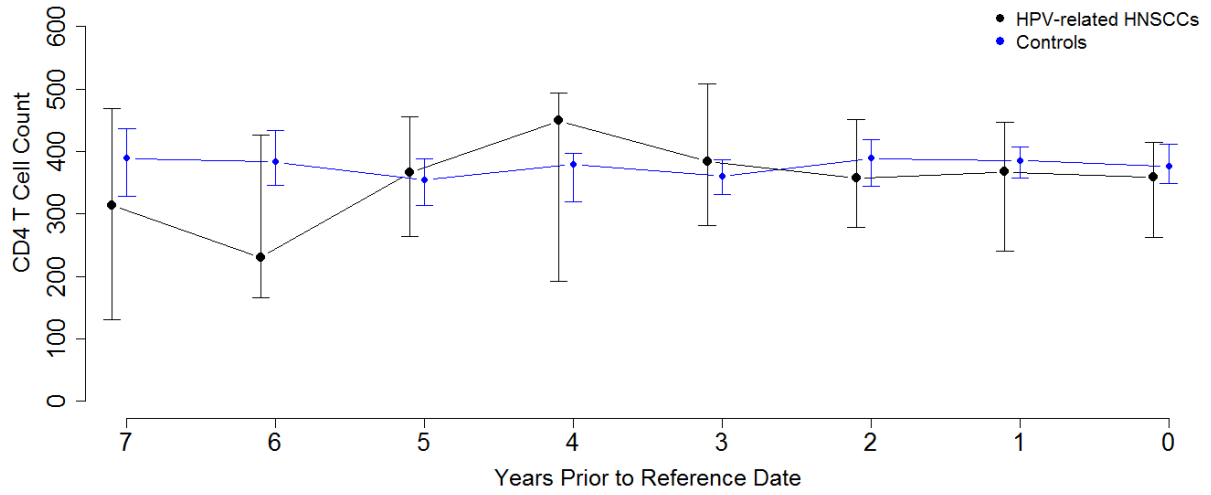
*p-interaction test is between cohort status and CD4 (350 cells/ul)
~Not adjusted for other risk factors
^unable to calculate due to the small number of females in cohort A

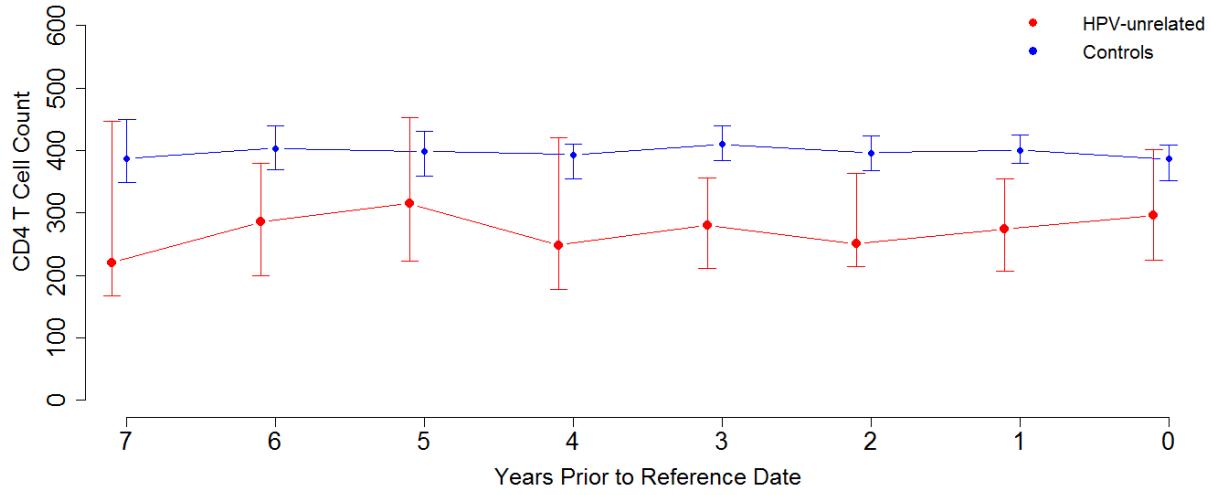
Table 4.5: The effect of risk factors on HPV-related and HPV-unrelated HNSCC among cohorts with available smoking data*

Immunosuppression Covariate	HPV-related HNSCC		HPV-unrelated HNSCC	
	IRR	aIRR^	IRR	aIRR^
Age				
<40	REF	REF	REF	REF
40-50	2.2 (0.81-6.1)	2.2 (0.80-6.2)	7.7 (3.1-19.0)	7.4 (3.0-18.4)
50+	3.1 (0.91-10.6)	3.2 (0.93-11.2)	14.0 (5.2-37.3)	13.8 (5.2-36.9)
Sex				
Female	REF	REF	REF	REF
Male	1.0 (0.36-1.8)	0.90 (0.32-2.5)	0.84 (0.37-1.9)	0.74 (0.32-1.7)
Baseline CD4				
≥500 cells/uL	REF	REF	REF	REF
200-500 cells/uL	1.8 (0.58-5.8)	1.7 (0.54-5.3)	4.6 (1.6-13.3)	4.2 (1.5-12.2)
<200 cells/uL	0.91 (0.23-3.6)	0.74 (0.18-3.0)	2.7 (0.85-8.8)	2.2 (0.69-7.2)

^ Poisson regression modeling that adjusted for age, gender, ever smoking status and cohort
*Excludes the largest available cohort – Cohort A

Figure 4.2**:** Median CD4 T cell counts measured before the reference date among HPV-related HNSCC cases, HPV-unrelated HNSCC cases, and matched controls in the NA-ACCORD





*Error Bars represent the 95% confidence intervals for each time point. The reference date was defined for HPV-unrelated and HPV-related HNSCC cases as the date of HNSCC diagnosis and for controls as the date after the same length of follow-up in the NA-ACCORD as that matched HNSCC case

Table 4.6: Impact of different measures of CD4 T cell count on HPV-related and HPV-unrelated HNSCC in the HIV-infected individuals in the NA-ACCORD

CD4 cell count measure	**HPV-related HNSCC**	**HPV-unrelated HNSCC**
	aOR^	**aOR^**
CD4 0-1 years before Cancer Diagnosis/Reference Date*		
≥500 cells/uL	REF	REF
200-500 cells/uL	1.8 (0.90-3.6)	1.7 (1.0-2.8)
<200 cells/uL	2.3 (1.1-4.8)	3.3 (2.0-5.6)
CD4 2-4 years before Cancer Diagnosis/Reference Date*		
≥500 cells/uL	REF	REF
200-500 cells/uL	1.2 (0.61-2.5)	1.6 (1.0-2.6)
<200 cells/uL	1.6 (0.73-3.6)	4.2 (2.6-7.2)
CD4, 5-7 years before Cancer Diagnosis/Reference Date*		
≥500 cells/uL	REF	REF
200-500 cells/uL	3.1 (1.0-9.5)	2.2 (1.1-4.1)
<200 cells/uL	5.1 (1.5-17.3)	4.2 (2.1-8.4)
CD4, 8-10 years before Cancer Diagnosis/Reference Date*		
≥500 cells/uL	REF	REF
200-500 cells/uL	1.7 (0.41-6.7)	1.5 (0.50-4.2)
<200 cells/uL	0.35 (0.03-6.7)	5.1 (1.8-15.0)

^10 controls were matched to each HNSCC cases and were matched on age (±5 years), cohort, sex, and enrollment year in the study. Using conditional logistic regression models, the association with each CD4 measure was considered separately and was adjusted for smoking status and current ART status.

*Controls were matched to cases utilizing incident density sampling, thus the reference dates for controls had the same amount of study follow-up time as the cancer diagnosis date for cases

Chapter 5 – Conclusions and Future Directions

Chapter 5: Conclusions and Future Directions

Summary of Results:

The focus of this dissertation was to investigate oral HPV infection and Head and Neck Squamous Cell Carcinoma (HNSCC), an understudied but emerging issue particularly in higher income countries.[1] In this dissertation, we concentrated on a group expected to have a particularly high burden of this disease, HIV-infected individuals.

The goals of this dissertation were 1) to compare the natural histories of oral HPV and anal HPV infection among HIV-infected individuals (chapter two), 2) to evaluate the effect of HIV-related immunosuppression along with other risk factors on oral HPV incidence and clearance in HIV-infected and HIV-uninfected individuals (chapter three), and 3) to examine the incidence, calendar trends and risk factors for HNSCC in HIV-infected individuals (chapter four).

In the second chapter of this dissertation, we utilized the Human Oral Papillomavirus Etiology (HOPE) study to explore the natural history of oral and anal HPV infections in 404 HIV-infected women, heterosexual men, and Men who have Sex with Men (MSM) in a HIV-related care clinic in Baltimore, MD. This study suggested that over half of the incident anal and oral HPV infections clear within two years and that there is a mix of persistent and intermittent infections in this immunosuppressed HIV-infected population.

We hypothesized that the incidence of anal HPV would be considerably higher than oral HPV, and that the persistence of both infections would be similar. We did indeed find that the incidence of anal HPV was five-fold higher than oral HPV infection, but we also noted the persistence of anal HPV was modestly higher than oral HPV even after adjustment for various risk factors. This suggests that HPV may be less transmissible to the

oral cavity and pharynx potentially due to a lower propensity for epithelial microtrama and possibly easier to clear due to unspecified immunologic features or other microbiota found in the oral region.[2] In addition, the higher incidence and persistence of anal compared to oral HPV infection likely partially explains why there is a much higher burden of HPV-associated anal cancer than HPV-associated HNSCC in HIV-infected individuals.[3,4]

Our second hypothesis involving the HOPE study was that HIV-infected MSM would have the highest hazard of incident anal HPV infection, while heterosexual men would have the highest hazard of incident oral HPV infection after adjusting for other factors such as the number of sexual partners. While both anal and oral HPV infections were detected in all three subgroups, anal HPV incidence was higher in MSM and women compared to heterosexual men, while oral HPV incidence was higher in heterosexual men than the women and MSM. This supports other evidence implying that anal HPV is often acquired through receptive anal intercourse, and suggests that oral HPV *may* be more likely to be acquired from performing oral sex on a woman (cunnilingus) than performing oral sex on a man (fellatio). However, it is also possible that heterosexual men may have a higher incidence of oral HPV due to their lower level of antibodies to the L1 capsid of HPV that may protect against subsequent oral HPV infection.[5-7]

In chapter three, we utilized two long term HIV-studies, the Multicenter AIDS Cohort Study (MACS) and the Women's Interagency HIV Study (WIHS), to evaluate the effect of HIV-infection, immunosuppression, and other risk factors on the incidence and clearance of oral HPV infection. Our nested study, the Persistent Oral human Papillomavirus Study (POPS), was able to evaluate these factors given its larger sample size of 1,230 HIV-infected and HIV-uninfected participants from the MACS and WIHS and strong retention rate.

Similar to the HOPE study, we found that over a quarter of HIV-infected and HIV-uninfected individuals in the POPS had a newly detected oral HPV infection within two years of follow-up, but the majority of these oral HPV infections cleared fairly rapidly. Counter to our original hypothesis, we did not observe an association between immunosuppression and reduced oral HPV clearance in the POPS. Instead, we observed a strong association between immunosuppression and increased oral HPV incidence. This suggests that the increased prevalence of oral HPV infection in HIV-infected individuals reported in previous cross-sectional studies [8-10] is likely explained by an increase in oral HPV incidence and not from a difference in oral HPV clearance.

The incident oral HPV infections detected in the POPS and HOPE studies were likely a combination of both newly acquired infections and re-activated infections. Oral HPV incidence was associated with a higher number of recent oral sex and rimming partners in HIV-uninfected individuals in the POPS, particularly among the small subset of individuals reporting that they recently performed oral sex on a woman. This suggests that some of these incident oral HPV infections were likely newly acquired. In addition, oral HPV incidence was associated with an increased lifetime number of oral sex partners, and we also observed a relatively high incidence of oral HPV infection in individuals reporting sexual abstinence throughout the POPS. This suggests that some newly detected infections are likely not newly acquired, but rather they are infections that were previously acquired and are being re-activated similar to what has been in observed in studies exploring cervical HPV.[11,12]

We also explored other risk factors for oral HPV clearance and incidence within the POPS. Clearance of oral HPV was lower in older compared to younger individuals, in current compared to never cigarette smokers, and in MACS men compared to WIHS

women. This supports other studies suggesting that tobacco may reduce local immune function,[13,14] and that women may have a stronger immune response to infectious diseases like HPV potentially due to endocrine differences.[15] In addition to HIV-related immunosuppression and increased sexual behavior, oral HPV incidence was also associated with never having a tonsillectomy, and a younger age.

In Chapter four of this dissertation, we followed North American HIV-infected individuals over time and examined the incidence and risk factors for HNSCC, a malignancy often caused by oral HPV infection. For this aim we included 82,375 HIV-infected individuals from the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD), which included 17 clinical and interval cohort studies who contributed data from the antiretroviral era between 1996 and 2009.

This study found that the age-standardized incidence of HPV-related and HPV-unrelated HNSCC were both approximately three times higher in the HIV-infected NA-ACCORD population compared to the general population (SIR=3.4, 95%CI=3.0-3.8). In addition, we found that age-standardized incidence of HNSCC in HIV-infected individuals was relatively constant during the ART era (1996-2009). However, there was a non-significant increase in HPV-related HNSCC when comparing the more recent era (2001-2010) to the early ART era (1996-2000) suggesting that HPV-related HNSCC may be modestly increasing similar to the general population.[1,16]

The incidence of HPV-related and HPV-unrelated HNSCC was higher in those with a low CD4 T cell count after adjustment for other risk factors. While results from the POPS and others have suggested that immunosuppression may have the strongest impact on the

earlier stages of the HPV-related carcinogenesis process,[17,18] we observed that low CD4 closer to cancer diagnosis may also have an impact on HNSCC incidence.

Public Health Implications

There are several implications to this research. This dissertation finds that both the prevalence of oral HPV infection and HPV-related HNSCC are modestly elevated in HIV-infected individuals, but is still a relatively less common malignancy in HIV-infected individuals particularly compared to other HPV-related malignancies such as anal cancer. We found that HPV-related HNSCC may be modestly increasing in HIV-infected individuals over time, while the incidence of HPV-unrelated HNSCC may be declining.

This dissertation research suggests that oral HPV is commonly detected in HIV-infected individuals, but most of these infections are cleared or controlled quickly even in the more immunosuppressed HIV-infected individuals. We also found that oral HPV is less commonly detected than anal HPV in HIV-infected individuals, and oral HPV is potentially cleared or controlled ever quicker than anal HPV. Therefore there are likely specific factors in the oral cavity and pharynx that can impact HPV that need more exploration. In addition, this suggests that a one-time measurement of an oral HPV infection, even oral HPV16, has a low specificity for subsequent HNSCC and is not useful in predication for this malignancy. This argues against utilization of oral HPV DNA alone as an oral cancer screening test.

The high number of intermittent infections and evidence of re-activation of latent infection seen in our studies suggest that immunosuppressed individuals in particular, may have difficultly continually controlling their oral HPV infections or may be more likely to re-acquire previously cleared oral HPV infections. However, it does appear that most individuals are able avoid continual persistence of oral HPV infection, regardless of their

CD4 T cell count. This suggests that immune re-constitution through utilization of antiretroviral therapy (ART) may be primarily beneficial in reducing the re-activation or the acquisition of oral HPV infection. However, considering the association between HPV-related HNSCC and reduced CD4 near cancer diagnosis, it is possible that immune competency could be beneficial at multiple stages in the natural history of this disease. This suggests that there may be an eventual reduction in the age-standardized incidence of HNSCC in HIV-infected individuals in developed countries. As HIV-infected individuals in developed countries are now initiating ART more quickly after their HIV diagnoses,[19] they will likely reduce their cumulative amount of time being immunosuppressed, which could reduce their subsequent risk for HNSCC.

Another important implication of this research is that heterosexual men may be at higher risk of oral HPV acquisition and subsequent HNSCC compared to MSM and heterosexual women. This may partially explain why the incidence HPV-related HNSCC isn't as common as some other HPV-associated cancers in the HIV-infected population of the United States given that heterosexual men and WSW likely only represent around 25% of this population.[19] However, the proportion of heterosexual men infected with HIV is considerably higher in lower income countries. Thus, it is possible that the incidence of HPV-associated HNSCC may be higher in HIV-infected individuals in low income countries. Exploration is warranted.

This research is also one of the first to report that both rimming and oral sex likely have roles in transmitting oral HPV infection. Thus, it implies that reducing certain sexual behaviors could reduce the likelihood of acquiring this oncogenic infection, although the co-linearity between these behaviors and the possibility of re-activation of oral HPV infection

makes it difficult to determine the exact risk of these behaviors. Finally, this dissertation's finding that oral HPV is still acquired in this middle aged population suggests that it is possible that the prophylactic HPV vaccines may have some potential benefit in populations outside the individuals currently recommended for vaccination (HIV-infected and HIV-uninfected individuals aged 11-26).[20,21]

Directions for Future research:

Based on the results of this dissertation, there are several different areas for future research. Future studies involving oral HPV infection should work to better understand: 1) how oral HPV is acquired, 2) the role of factors specific to the oral region such as oral antibodies, and 3) the role of gender on infection and cancer incidence. In addition, studies including HNSCCs should further explore immunosuppression's role in HPV's carcinogenesis process, and examine the proportion of HNSCCs that are positive for HPV particularly in HIV-infected individuals. Finally, evaluation of potential preventative measures such as HPV vaccination and potential screening markers in higher risk populations are warranted.

While this dissertation suggests oral sex and rimming are transmitters of oral HPV infection, further work is needed to better elucidate how oral HPV is acquired considering the considerable number of infections not explained by oral sex or rimming behavior. Prospective studies including younger partners are warranted to explore the role of deep (French) kissing, autoinoculation, and other potential ways to transmit this virus.

We also found that the natural history of oral HPV infection may differ from anal HPV. I am involved with others in conducting further comparisons between oral HPV and anogenital HPV infection in studies such as the MACS and WIHS. There is still a need to

better understand factors specific to the oral region that could impact the natural history of oral HPV and HPV-associated HNSCC. Comparing HPV at different anatomical sites and exploring factors that could modify the risk of disease such as a history of tonsillectomy, oral specific antibodies (IgG and IgA), and oral microbiota are worthy of further exploration.

Another area of further interest is exploring the differences in the natural history of oral HPV and HNSCC by gender. Recent studies have suggested that prevalence of oral HPV infection is higher in males,[22] and that HPV-associated HNSCC is around three times as prevalent in males compared to females.[1,16] However, the reasons for male predominance of HPV-associated HNSCC are undetermined. As previously mentioned, this dissertation provides some modest evidence that this difference may be at least partially due to a difference in transmission risk between sexual acts. It still undetermined whether cunnilingus may be more likely to transmit oral HPV than fellatio due to a higher viral load in the vagina, or whether the difference may be due to differing prevalence's of natural protective antibodies to the L1 capsid protein.[23] It is possible that the male predominance of HPV-associated HNSCC may also be due to a difference in oral HPV persistence, as women may clear their infections more quickly due to gender related endocrine differences. [15] These hypotheses need further investigation in the more general HIV-uninfected population. Considering that females have a more robust immune response to many infectious diseases and that males more commonly develop cancer at shared anatomic sites,[24] this research could be extended to other cancers and considered in future preventative strategies.

While this dissertation found that immunosuppression may primarily act on the earliest stages of carcinogenesis (oral HPV incidence), we also found that immunosuppression may play a role near HNSCC diagnosis. Thus there is still a need to

better understand the timing of immunosuppression and how it can impact cancer etiology and survival particularly in populations such as the HIV-infected and organ transplant recipients. A better understanding of the role of inflammation and/or immunosurveillance on HPV-related HNSCC and other malignancies is necessary. This is especially important for HIV-infected individuals as it could help inform when antiretroviral therapy could be best initiated in terms of cancer prevention.

Oral HPV infection has been detected in approximately 70% of oropharyngeal cancers in the general population of the United States,[1] however it is undetermined what proportion of oropharyngeal cancers and other HNSCCs in HIV-infected individuals are caused by oral HPV. Other oncogenic HPV types besides HPV16 are more commonly detected in HIV-infected individuals,[8,25] thus other types should be tested for and considered in HNSCC tumors. This would be particularly enlightening given that currently the only oncogenic HPV types that the prophylactic HPV vaccines protect against are HPV16 and HPV18.

Further evaluation of the HPV vaccine efficacy in HIV-infected individuals is warranted. Initial studies suggest that the vaccine is safe and immunogenic in this population,[26] and several on-going trials are further exploring this question.[27] Recent observational studies have suggested that cervical pre-cancerous lesion recurrence is lower among individuals vaccinated after their first excision/conisation.[28] Thus, while the prophylactic HPV vaccines have shown to protect against newly acquired infection, it is unclear, but possible, that vaccination may also protect against re-acquisition or re-activation of oral HPV infection. The high incidence of oral HPV infection in this dissertation coupled with the high level of cervical and anal disease burden in HIV-infected individuals may

support vaccinating these individuals older than 26. However, further study from randomized control trials is necessary.

Finally, future studies should consider the efficacy of cancer screening markers for HPV-associated HNSCC in the HIV-infected population. A recent nested case-control study suggested that antibodies to HPV16 E6 are very strongly associated with HNSCC detected ten or more years later (OR=274, 95%CI=110-681).[29] Thus, we are now conducting a study exploring whether antibodies to this oncoprotein are detectable in the cancer free POPS population who have persistently detected oral HPV16 DNA. A beneficial screening test for HPV-associated HNSCC is difficult to develop considering HNSCC is a relatively uncommon malignancy, even among higher risk groups. Thus screening markers such as HPV16 E6 would require an extremely high specificity, and should not be recommended until further validation from randomized control trials with mortality as the end-point.

Conclusions:

This dissertation examined the natural history of oral HPV infection and HNSCC among HIV-infected individuals. We present two of the first studies exploring the natural history of oral HPV infection, and find that oral HPV is commonly detected in this HIV-infected population, but the majority of these infections clear quickly. We also contributed to the body of research that suggests that HPV-related HNSCC is modestly elevated in HIV-infected individuals, but it still not a common malignancy in this population and not rapidly increasing in the ART era. We find that immunosuppression may have the strongest effect on oral HPV incidence as it may lead to increased risk of both newly acquired and re-activation of latent oral HPV infection. Additionally, we suggest that HIV-infected heterosexual men may be at higher risk of newly acquired infection and subsequent

malignancy. Future research is necessary to better understand the impact of risk factors including HIV and immunosuppression on oral HPV and HNSCC. Additionally, research on preventative methods such as HPV vaccination and exploration of screening markers in this population are warranted.

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Appendix A: Lack of impact of HPV16 seropositivity on the subsequent risk of oral HPV16 infection

Lack of impact of HPV16 seropositivity on the subsequent risk of oral HPV16 infection

Abstract:

Introduction: Previous studies have suggested that Immunoglobulin G (IgG) antibodies to virus like particles (VLP) to the HPV L1 capsid protein acquired through a natural infection may protect against the subsequent incidence of cervical HPV infection. However, it is underdetermined how HPV VLP seropositivity may impact the risk of oral HPV infection.

Methods: Serum samples from 476 HIV-infected and 297 at risk HIV-uninfected participants were tested for antibodies at study baseline to virus-like particles to the L1 capsid protein of HPV16 using an enzyme linked immunosorbent assay (ELISA). Oral rinse samples were collected at semi-annually follow-up visits for up to three years. DNA was isolated from the oral rinse and tested using PGMY 09/11 primers. Adjusted Poisson and Wei-Lin-Weissfeld regression modeling were utilized.

Results: 23% of HIV-infected and 20% of HIV-uninfected individuals were HPV16 seropositive. HPV16 seropositivity was not associated with baseline oral HPV16 DNA detection (aPR=0.50, 95%CI=0.18-1.4), but was associated with baseline anal HPV16 DNA detection among the MACS participants (aPR=2.3, 95%CI=1.5-3.7). The risk of incident oral HPV infection during follow-up was similar comparing individuals who were HPV16 seropositive compared to those who were HPV16 seronegative at baseline (aHR=1.2, 95%CI=0.43-3.3).

Conclusions: Oral HPV may be less likely to induce a natural antibody response compared to anal HPV. Natural HPV16 seropositivity does not appear to reduce the subsequent risk of oral HPV16 infection.

Introduction:

While HPV infection is commonly detected in the genital tract, these infections often clear or are controlled within twelve months.[1,2] These transient anogenital infections have been shown to often induce a natural immune response to virus-like particles (VLPs) from the L1 capsid protein of HPV. These natural developing serum immunoglobulin G (IgG) antibodies have been suggested to be a surrogate measure for cumulative lifetime exposure to HPV, [3-6] although they have limited sensitivity as there are some individuals who are exposed to HPV DNA that do not seroconvert. Further, some initial studies suggest people with natural HPV antibodies may reduce the risk of subsequently acquiring a new cervical HPV infection of that type [7,8] although other studies have not seen an association.[9,10]

Serum IgG antibodies generated in response to a genital HPV infection could potentially protect not only against subsequent anogenital infection but also against acquisition of subsequent oral HPV infection. However, as there have only been a few studies exploring the natural history of oral HPV, whether natural serum VLP antibodies may provide protection against subsequent risk of incident oral HPV infections has not been explored. Oral HPV16 infections are of particular interest as they are the mostly commonly detected type in the oral region,[11,12] and are known to cause over 85% of HPV positive Head and Neck Squamous cell carcinomas (HNSCC).[13]

We utilized a longitudinal cohort study of HIV-infected and at risk HIV-uninfected individuals expected to have a high HPV seroprevalence to VLPs to explore the relationship between HPV16 seropositivity and subsequent risk of oral HPV16 infection.

Methods:

Study Participants

For this study, 773 individuals were selected from the Persistent Oral human Papillomavirus Study (POPS). The POPS includes HIV-infected and at risk HIV-infected participants from the Multicenter AIDS Cohort Study (MACS) and the Women Interagency HIV Study (WIHS), and is further described elsewhere.[11,14,15] All participants that enrolled into the POPS between 2009 and 2010 and had four or more follow-up visits at the time of this sample selection for antibody testing were included. The executive committees of the MACS and WIHS and the Institutional Review Boards from each site approved the study protocol. All POPS participants provided written informed consent.

Banked serum was collected from each individual's baseline POPS visit and tested for HPV16 VLP antibodies. This data was linked to the oral HPV results and risk factor information at the baseline POPS visit and every six month follow-up visit as previously described in chapter three of this dissertation. In addition, anal swab samples collected from MACS participants at the around the same time as the POPS baseline visit were evaluated.

Laboratory testing:

Antibody testing was performed on banked serum samples at the POPS baseline by using a virus-like particle (VLP)-ELISA with HPV 16 capsids produced in insect cells from recombinant baculoviruses following previously published methods with some modifications (VLP production[6]; ELISA[16]). For quality assurance, known positive controls were run on each 96 well ELISA plate throughout the testing period. For any run where the controls gave optical density (OD) values outside of an acceptable limit, the plate was re-run.

Oral HPV16 DNA was collected through a 30 second rinse and gargle with saline or Scope™ mouthwash.[17] In the MACS, anal swab specimens were also collected and tested for anal HPV DNA. The DNA from the anal swabs was collected using a saline-moistened

Dacron swab that was inserted six cm into the anal canal and removed in a circular motion against the anal wall. The swab was placed in 1 mL of Sample Transport Medium (Digene Diagnostics, Silver Spring, MD).[18] DNA was isolated from these samples using a magnetic bead-based automated platform (QIAsymphony SP, Qiagen),[19] and then tested for the 37 different HPV types utilizing the Roche linear array with PGMY09/11 primers PCR primer pools and reverse line blot hybridization similar to chapter 3 of this dissertation and other previous studies which further describes these assays.[11,17,19]

Statistical methods

To compare baseline HPV16 seroprevalence between various risk factors, we utilized Chi-square tests for categorical variables and Mann-Whitney tests for medians for continuous variables. Individuals were considered seropositive when the optical density of the test was above a cutpoint of 0.2 (determined based on previous studies).[6] Optical density was also considered as a continuous variable in these analyses.

As previously mentioned in chapters 2 and 3, type specific oral HPV infection was classified as prevalent if detected at baseline and as incident if first detected after a negative type-specific test at baseline. Unadjusted Poisson regression with robust variance was used for analyzing risk factors for baseline HPV16 seroprevalence. Unadjusted and adjusted Wei-Lin-Weissfeld (WLW) models were utilized to explore whether HPV16 seroprevalence may impact subsequent oral HPV incidence. These marginal models involve Cox proportional hazard assumptions with an exchangeable correlation structure and robust variance to adjust for multiple infections with a single individual.[20] All statistical tests were two sided and considered significant using an alpha=0.05 level. All analyses were performed by STATA MP Version 12.0 (STATA Corp, College Station, TX).

Results:

Among the 773 participants who contributed four or more study visits to the POPS, there were 174 (22%) who were seroprevalent for HPV16 VLP at the POPS baseline visit. HPV16 L1 seropositivity was similar by HIV-status (HIV+ vs. HIV-: 23% vs. 20%, p=0.25), gender (male vs. female: 23% vs. 21%, p=0.36), and age (Table A.1). In unadjusted analysis, higher seroprevalence of HPV16 VLP was observed in those with a current CD4 T cell count<200 cells/uL, in never smokers, and in those with two or more recent oral sex partners (Table A.1, all p<0.05). After adjustment with other risk factors, never smoking cigarettes, increased number of recent oral sex partners, and reduced current CD4 T cell count all remained associated with increased HPV16 VLP seroprevalence (Table A.1, all p<0.05). When optical density values were considered continuously, HPV16 antibody titer levels were similar between HIV-infected and HIV-uninfected individuals (Figure A.1).

We compared the baseline HPV16 seropositivity to the prevalence of anal and oral HPV DNA at baseline (Table A.2). We observed that HPV16 seropositivity was associated both any anal HPV prevalence at baseline (PR=1.2, 95%CI=1.1-1.4) and anal HPV16 DNA prevalence at baseline (PR=2.3, 95%CI=1.5-3.7). In addition, when HPV16 antibody titer was considered as a continuous variable, higher antibody titer remained strongly associated with baseline anal HPV16 DNA prevalence (p-trend=0.007, Table A.2). In contrast, HPV16 seropositivity was not associated with either oral HPV prevalence at baseline (PR= 1.1, 95%CI=0.88-1.4) or oral HPV16 DNA prevalence at baseline (PR=0.50, 95%CI=0.18-1.4, Table A.2). Only 13% of individuals who were oral HPV16 positive at baseline were HPV16 seroprevelant, while a larger proportion (43%) of those were anal HPV16 positive at baseline were HPV16 seroprevelant at baseline (p=0.004).

Baseline seropositivity to HPV16 VLP did not reduce subsequent risk of any oral HPV infection (aHR=1.0, 95%CI=0.76-1.3) or oral HPV16 infection (aHR=1.1, 95%CI=0.41-3.0) in this study (Table A.3). Similarly there was no observed association when considering the antibody titer's optical density continuous or by quartile (Table A.3). Seropositivity did not reduce subsequent oral HPV16 risk in either HIV-infected (aHR=0.92, 95%CI=0.23-3.6) or HIV-uninfected (aHR=2.2, 95%CI=0.56-8.3) individuals. When restricting to individuals who reported that they performed oral sex during the study, seropositivity to HPV16 VLP at baseline remained unassociated with subsequent risk of oral HPV16 infection (HR=3.1, 95%CI=0.74-13.1, Table A.3).

Discussion:

This study suggests that HPV16 seropositivity does not have a protective effect on subsequent acquisition of oral HPV infection. In addition, we find that natural HPV16 antibody responses may be elicited more frequently by anogenital infections than oral infections, as HPV16 seroreactivity was associated with anal but not oral HPV16 DNA prevalence detected at the same baseline visit.

This study is one of the first to examine whether natural antibody response may be protective against subsequent oral HPV infection. Similar to two other cervical HPV studies,[9,10] we do not observe any protective effect of higher HPV16 antibody titer on subsequent risk of oral HPV16 infection. However, this contrasts with several other previous cervical HPV studies, which suggested that high HPV16 antibody titer from natural infection can reduce subsequence cervical HPV infection risk, [7,8] including a large recent study that suggested that the risk of subsequent infection was halved in those with high

HPV16 antibody tier.[7] It is unclear whether antibody response may have a differential impact on anogenital HPV infection compared to oral HPV infection risk.

As suggested in chapter 3 in this dissertation, the incident oral HPV infections detected in the POPS were often intermittent and may represent reactivated infection rather than newly acquired infection particularly in HIV-infected individuals. Thus, the high number of re-activated infection may have impacted these results; however that was still no evidence of protection when restricting to individuals recently performing oral sex or among HIV-uninfected individuals alone.

Previous studies report that HPV16 seroprevalence is considerably higher in both women and MSM compared to heterosexual males, potentially due to their exposure to HPV at mucosal sites (anus/cervix) compared to heterosexual male's non-mucosal site (genitals) .[21,22] Thus, one proposed explanation of the male predominance of oral HPV infection and HPV-positive oropharyngeal cancer is that the higher HPV seroreactivity in women (and MSM) may protect them from subsequent oral HPV infection.[23] However, results from this study do not support this hypothesis. This suggests the higher incidence of oral HPV infection among heterosexual males may be due other factors such a potential higher risk of oral HPV transmission through cunnilingus compared to fellatio.[24]

The antibody titer response to natural HPV infection is considerably lower than those conferred from HPV vaccination through the Gardisil or Cervarix vaccines.[7,25,26] While formal clinical trials have not been performed, a recent study does suggest that the HPV vaccine may provide protection against subsequent oral HPV infection, in addition to the protection it confers against anogenital HPV infection.[27]

Our study's lack of association between baseline oral HPV DNA and HPV seropositivity is consistent with a cross-sectional study that also did not observe an association between oral HPV 16/18/33 DNA prevalence and HPV16/18/33 seropositivity in women. [22] However, we did observe an association between baseline *anal* HPV DNA and HPV seropositivity, suggesting that natural HPV16 antibody responses may be more likely conferred by anogenital infections. It is possible, that oral HPV may be less likely to induce an immune response because the viral loads of these infections are often lower than anogenital infections (Beachler unpublished).

HPV seropositivity has been associated to anogenital HPV prevalence in some,[28,29] but not all studies[6] as seropositivity is more likely to be associated with past rather than current HPV16 DNA positivity.[30] Thus further explanation needs to be explored on whether past oral HPV DNA may confer a subsequent antibody response similar to anogenital HPV infection.

There are several limitations and strengths to this study. The ELISA assay is limited as there are no standard reference serum samples and the assay has been suggested to only detect an antibody response only 50-60% infections.[22,29] In addition, this study population has reduced generalizability to the general population. However, this is one of the first studies to explore the effect of HPV16 antibody response on subsequent incidence of oral HPV16 and utilizes a well-established cohort study, and robust laboratory and statistical methods. [19,20]

This study suggests that natural HPV16 seroprevalence may not protect against the subsequent acquisition of oral HPV16 infection in a higher risk cohort. Further studies are

necessary to verify this result and explore whether serum antibody response may differ by the infection's anatomical site.

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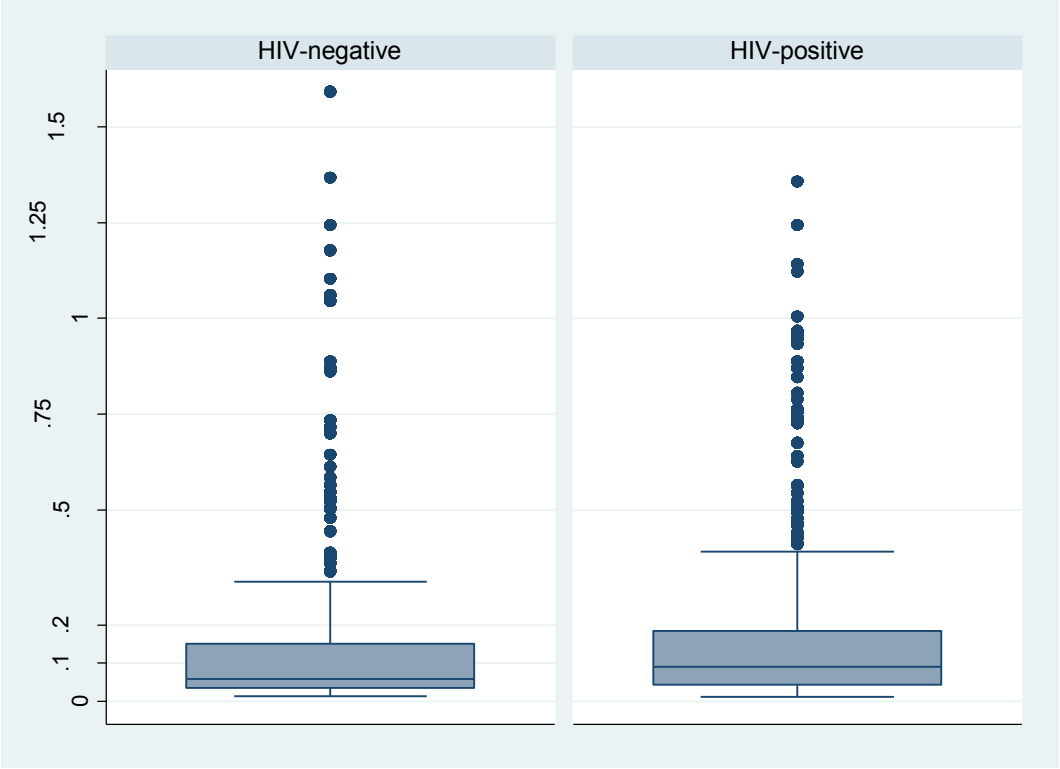
Table A.1: Characteristics of the 773 POPS serology participants by HPV16 VLP serostatus in unadjusted and adjusted modeling

Characteristics of POPS participants	HPV16 Seropositive n=174	HPV16 VLP Serology	
		Unadjusted (PR)	Adjusted (PR)
Age*			
Younger than 45	27%	REF	REF
45-55	20%	0.70 (0.47-1.1)	0.68 (0.43-1.1)
55 or older	20%	0.69 (0.45-1.1)	0.64 (0.38-1.1)
p-trend		0.32	0.34
Gender			
Female (WIHS)	21%	REF	REF
Male (MACS)	23%	1.2 (0.84-1.6)	1.2 (0.57-2.5)
Cigarette Smoker*			
Never	28%	REF	REF
Former	19%	**0.58 (0.37-0.90)**	**0.60 (0.38-0.96)**
Current	20%	**0.62 (0.41-0.91)**	**0.65 (0.41-1.0)**
Recent oral sex partners			
0	18%	REF	REF
1	24%	1.4 (0.94-2.2)	1.6 (1.0-2.6)
2 to 5	30%	**1.9 (1.2-3.0)**	**2.2 (1.3-3.8)**
6 or more	32%	**2.1 (1.1-3.9)**	**2.3 (1.0-5.1)**
p-trend		0.10	0.26
Lifetime number of oral sex partners			
0-4	20%	REF	REF
5 to 99	22%	1.1 (0.74-1.7)	1.0 (0.61-1.6)
100 or more	26%	1.4 (0.90-2.2)	0.92 (0.48-1.8)
p-trend		**0.03**	0.25
HIV-infection			
No	20%	REF	REF
Yes	23%	1.2 (0.86-1.8)	1.4 (0.96-2.1)
HIV-status + CD4 T cell count*			
Negative	20%	REF	REF
Positive CD4>500 cells/μL	23%	1.2 (0.83-1.9)	1.4 (0.89-2.1)
Positive CD4 200-499 cells/μL	21%	1.1 (0.69-1.7)	1.3 (0.79-2.1)
Positive CD4<200 cells/μL	33%	2.0 (0.98-4.2)	**3.0 (1.4-6.6)**
p-trend		0.18	**0.03**

Figure A.1: The distribution of the optical density of HPV16 Antibody Titer among the 773 HIV-negative and HIV-positive participants from the POPS.



*The difference in OD values between HIV-negative and HIV-positive participants were not significantly different in this study ($p>0.05$).

Table A.2: HPV16 serology relationship to oral and anal HPV16 and any HPV DNA at baseline of POPS

	Unadjusted (PR)			
	Oral HPV16 DNA	Oral HPV DNA	Anal HPV16 DNA	Anal HPV DNA
HPV16 VLP Seropositive				
No	REF	REF	REF	REF
Yes	0.50 (0.18-1.4)	1.1 (0.88-1.4)	**2.3 (1.5-3.7)**	**1.2 (1.1-1.4)**
HPV16 VLP antibody titer				
Q1 (lowest)	REF	REF	REF	REF
Q2	2.2 (0.78-6.3)	1.0 (0.78-1.4)	1.6 (0.42-6.2)	**1.1 (1.0-1.1)**
Q3	2.2 (0.78-6.2)	1.3 (0.98-1.7)	**4.2 (1.3-13.6)**	**1.3 (1.2-1.3)**
Q4 (highest)	1.0 (0.30-3.5)	1.2 (0.94-1.6)	**5.9 (1.9-18.8)**	**1.4 (1.4-1.5)**
continuous p-trend	0.45	0.10	**0.007**	**<0.001**

Table A.3: Baseline Seroprevalence and subsequent risk of oral HPV infection in the POPS

	Oral HPV16 incidence		**Oral HPV incidence**	
Measure	**Unadjusted HR**	**Adjusted HR^**	**Unadjusted HR**	**Adjusted HR^**
HPV 16 VLP seropositive				
No	REF	REF	REF	REF
Yes	1.4 (0.60-3.4)	1.1 (0.41-3.0)	1.2 (0.87-1.5)	1.0 (0.76-1.3)
HPV16 VLP antibody titer				
Q1 (lowest)	REF	REF	REF	REF
Q2	1.0 (0.26-4.2)	1.1 (0.23-5.2)	1.2 (0.80-1.8)	1.0 (0.70-1.5)
Q3	2.3 (0.67-7.6)	2.2 (0.60-7.9)	1.4 (0.97-2.1)	1.1 (0.77-1.7)
Q4 (highest)	1.8 (0.52-5.9)	1.4 (0.37-5.5)	1.4 (0.99-2.0)	1.1 (0.79-1.6)
continuous p-trend	0.39	0.78	0.21	0.45
HPV16 seropositivity restricted to those performing oral sex				
No	REF	REF	REF	REF
Yes	2.5 (0.82-7.4)	3.1 (0.74-13.1)	1.5 (1.0-2.6)	1.4 (0.99-2.1)

^adjusted for HIV-status, current CD4 T cell count, age, cigarette smoking, study site, history of tonsillectomy, recent tooth brushing, current alcohol use, lifetime and recent number of oral sex partners, and recent oral sex on a woman

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Doctor of Philosophy (PhD) in Epidemiology
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Master of Health Science (MHS) in Epidemiology (Cancer Option)
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Bachelor of Science (BS) in Bioengineering
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Institute for Training in Biostatistics -Sponsor: National Heart, Blood and Lung Institute
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Johnson & Johnson and the Johns Hopkins University Scholars Program, 2010-2013
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Professional Activities:

Society Membership and Leadership:
American Association for Cancer Research (AACR), 2010-present
Society for Epidemiologic Research (SER), 2010-present
American Public Health Association (APHA), 2012-present
Organizer, Cancer Epidemiology Journal Club, 2009-2010
Organizer, Epidemiologic Doctoral Student Representative 2010-2011
Organizer, Sexually Transmitted Infections Journal Club, 2011-2012
Student Funding Cabinet, Epidemiology Student Organization, 2011-2013

Honors and Awards:

- Harvey Meyerhoff Fellowship in Cancer Prevention – 2010 Recipient
- Academic scholarship from JHSPH Dept. of Epidemiology received, 2009-2010
- Delta Omega Honorary Society in Public Health Member, 2010-present
- Doctoral thesis research fund award – 2012 Recipient

Publications:

Peer Reviewed Journal Articles:

Beachler DC, Sugar EA, Weber KM, Margolick JB, Strickler HD, Cranston RD, Burk RD, Wiley DJ, Minkoff H, Reddy S, Gillison ML, D'Souza G. *High level of oral HPV acquisition, re-activation, and clearance, among HIV-infected and HIV-uninfected adults.* In Preparation.

Beachler DC, Abraham AG, Jing Y, Fakhry C, Silverberg MJ, Gill MJ, Dubrow R, Kitahata M, Klein M, Burchell AN, Moore RD, D'Souza G, and North American AIDS Cohort Collaboration on Res. and Design (NA-ACCORD) of IeDEA. *Incidence and Risk Factors of HPV-related and HPV-unrelated Head and Neck Squamous Cell Carcinoma in HIV-infected Individuals*. In Preparation.

Beachler DC, D'Souza G, Sugar EA, Xiao W, Gillison ML. (2013) *Natural history of anal versus oral HPV infection in HIV-infected men and women.* JID. 208(2):330-9. PMID: 23596319

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Beachler DC, Gellert L, Ambinder RF, Jacobson LP, Breen EC, Martinez-Maza O, Rabkin CS, Kaslow RA, D'Souza G. (2010) *Kaposi sarcoma-associated herpesvirus (KSHV) serum DNA and antibodies not associated with subsequent non-Hodgkin lymphoma (NHL) risk.* JAIDS. 56(2):188-92. PMID: 21116187

Invited Commentary:

Beachler DC, D'Souza G. *Natural history of oral papillomavirus infection in men.* The Lancet. 2013. 382(9895):839-41 PMID: 23852381

Beachler DC, D'Souza G. *Oral HPV infection and head and neck cancers in HIV-infected individuals.* Curr Opin in Onc. 2013. 25(5):503-10. PMID: 23827091

Beachler DC, D'Souza G. *Nuances in the Changing Epidemiology of Head and Neck Cancer.* Oncology. 2010. 24(10):924, 926. PMID: 21138173

Teaching Experience:

Tutor in the Epidemiology Department
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- 340.751 Epidemiological Methods 1
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Research Grant Participation:

- Fellowship in Sexual Transmitted Infections (STIs) – National Institutes of Health (NIH), T32 training grant, *2010-2012*
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Poster Presentations:

- Oral human Papillomavirus infection among HIV infected and uninfected men who have sex with men. American Association of Cancer Research, Washington DC, 2010.
- Kaposi's sarcoma-associated herpesvirus (KSHV) serum DNA not associated with subsequent non-Hodgkin's lymphoma (NHL) risk. International Conference on Malignancies in AIDS and Other Acquired Immunodeficiencies (ICMAOI), Bethesda, Maryland, 2010.
- Oral HPV prevalence and persistence are elevated among HIV-positive adults. International Papillomavirus Conference (IPV), Berlin, Germany, 2011.
- A community-academic partnership to prevent childhood obesity in Latino children in northern Philadelphia: a multifaceted program involving after-school physical activity, nutrition education, and workshops with caregivers, American Public Health Association (APHA) Meeting and Exposition, San Francisco, California, 2012.
- Differences in Anal and Oral HPV Infection among HIV-Infected Individuals – A Multi-Year Natural History Study. International Papillomavirus Conference, San Juan, Puerto Rico, 2012.

Invited Oral Presentations:

- Role of Oral HPV in Oropharyngeal Squamous Cell Carcinoma. Society of Otorhinolaryngology and Head-Neck Nurses Conference, Baltimore, MD, 2011.
- High Prevalence of Oral HPV Infection in HIV-positive and HIV-negative adults. Society for Epidemiologic Research (SER), Montreal, QC, 2011.
- Differences in Anal and Oral HPV Infection among HIV-Infected Individuals – A Multi-Year Natural History Study. Plenary Session - International Papillomavirus Conference, San Juan, Puerto Rico, 2012.
- Natural History and Risk Factors for Oral HPV Infection in HIV-infected and HIV-uninfected men and women. European Research Organization on Genital Infection and Neoplasia (EUROGIN) 2013, Florence, Italy, 2013.
- Oral HPV Infection, Persistence, and Natural History, European Research Organization on Genital Infection and Neoplasia (EUROGIN) 2013, Florence, Italy, 2013.
- HIV-Related Immunosuppression is Associated with Higher Incidence of Oral HPV, but Not Oral HPV Clearance. International Conference on Malignancies in AIDS and Other Acquired Immunodeficiencies (ICMAOI), Bethesda, Maryland, 2013.
- Role of Oral HPV in Oropharyngeal Squamous Cell Carcinoma. Southwest Region of the National Dental Practice-Based Research Network Annual Meeting, San Antonio, Texas, 2014.

Community Service:

Peer Review Journal Reviewer: Journal of Acquired Immune Deficiency Syndromes (JAIDS), Journal of Infectious Disease (JID), Oral Oncology (OO), Emerging Infectious Diseases (EID), Cancer Epidemiology

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